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**DEPARTMENT OF DEFENSE  
ANIMAL CARE AND USE PROGRAMS 2001**

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## LIST OF ACRONYMS

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AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
AALAS	American Association for Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
ACUP	Animal Care and Use Program
APHIS	Animal and Plant Health Inspection Service
ASBREM	Armed Services Biomedical Research Evaluation and Management
AWA	Animal Welfare Act
CHPPM	Center for Health Promotion and Preventive Medicine
DARPA	Defense Advanced Research Projects Agency
DoD	Department of Defense
DTIC	Defense Technical Information Center
FDA	Food and Drug Administration
FY	Fiscal Year
GEIS	Global Emerging Infections Surveillance and Response System
GME	Graduate Medical Education
IACUC	Institutional Animal Care and Use Committee
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILAR	Institute of Laboratory Animal Research
LAM	Laboratory Animal Medicine
NHP	Nonhuman Primate
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
OSD	Office of the Secretary of Defense
PL	Public Law
RDT&E	Research, Development, Test, and Evaluation
SBCCOM	Soldier and Biological/Chemical Command
SPF	Standard Protocol Format
USAMRICD	U.S. Army Medical Research Institute of Chemical Defense
USDA	United States Department of Agriculture
USUHS	Uniformed Services University of the Health Sciences
VEE	Venezuelan Equine Encephalitis
WRAIR	Walter Reed Army Institute of Research

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# SECTION I

## INTRODUCTION/OVERVIEW

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The Fiscal Year (FY) 2001 Report on Department of Defense Animal Care and Use Programs was conducted by the Director, BioSystems, Office of Director, Defense Research and Engineering. In addition to a general overview, this report provides a summary of Department of Defense (DoD) animal use with respect to research and training activities. It also addresses the underlying rationale, or benefits, of this animal use and efforts by the DoD to implement animal use alternatives.

### I.1 DoD POLICY GOVERNING ANIMAL RESEARCH

The DoD is committed to full ethical and regulatory compliance for its animal-based research programs. It has been proactive in increasing the fixed infrastructure and span of control necessary to ensure compliant, responsible, and efficient execution of programs and maximize oversight of diverse and varied missions. The Department has aggressively implemented focused programs and policy documents that optimize standardization of animal care. This enhanced standardization and oversight have improved a historically good system and made it outstanding and a model to be emulated.

In 1995, the DoD revised and implemented the directive dealing specifically with animal care and use ([DoD Directive 3216.1, “Use of Laboratory Animals in DoD Programs,” 1995](#)). This directive strengthens and clarifies requirements for nonaffiliated membership in institutional oversight and directs all DoD animal use facilities that maintain animals for RDT&E and training to apply for AAALAC accreditation. DoD veterinarians, researchers, and policymakers continue in their efforts to be proactive in maintaining the highest level of accountability over animal use.

The DoD also implemented a Policy Memorandum entitled [“Department of Defense \(DoD\) Policy for Compliance with Federal Regulations and DoD Directives for the Care and Use of Laboratory Animals in DoD-Sponsored Programs.”](#) This 1995 Policy Memorandum specifies training requirements for nonaffiliated DoD Institutional Animal Care and Use Committee (IACUC) members, implements a [standard format for animal use protocols](#), a [standard checklist for IACUC inspections](#), and a standard reporting requirement for all animal use research to support the [Biological Research Database](#), which is publicly accessible. All animal research must conform to requirements of the 1966 Animal Welfare Act (AWA) (Public Law [PL] 89-544) as amended in 1970 (PL 91-579), 1976 (PL 94-279), 1985 (PL 99-198), and 1990 (PL 101-624) as well as the National Research Council’s Guide for the Care and Use of Laboratory Animals, (7th rev. edition, 1996), the [U.S. Government Principles for Animal Use \(1985\)](#), and the requirements of the applicable regulations of the United States Department of Agriculture (USDA).

Mice and rats are the most commonly used species in research. Although the AWA and its implementing regulations currently exempt these species, the DoD has long afforded them, along with all other vertebrates, including fish and frogs, the same consideration given nonexempt species under the AWA. In implementing a full accounting of the use of mice and rats, the DoD is relatively unique in the scientific research community. At the same time, DoD researchers have aggressively developed novel procedures to replace, reduce, and refine the use of animals in their research.

### I.2 REQUIREMENTS NECESSITATING THE USE OF ANIMALS BY THE DoD

The DoD use of animals in research, development, test, and evaluation (RDT&E) and in education and training programs is critical to sustained technological superiority in military operations in defense of our national interests. These DoD programs that are dependent upon animal use ultimately translate into improved military readiness as well as reduction in morbidity and mortality associated with military operations. Many of these programs directly contribute to Force Health Protection, allowing our forces to operate in and survive the

numerous and various hazards they face around the world. Researchers in the DoD are committed to accomplishing this goal, and it is important to emphasize that, as in nonmilitary research programs, the involvement of animals in research cannot always be avoided.

The DoD research has benefited greatly from animal use alternatives such as nonliving systems, cell and tissue culture, and computer technology. However, complex human organ system interactions, in addition to environmental factors and confounding variables, necessitate the continued judicious use of animal models in DoD programs. Although many innovative animal use alternatives have been developed and are in use by Department scientists, situations remain for which there are no acceptable nonanimal alternatives. The DoD continues to embrace new advances, technologies, and breakthroughs in animal use alternatives. Section III of this report provides a summary of the many animal use alternatives being explored and implemented in DoD laboratories.

Disease remains a major cause of disability and sometimes death in military operations and conflicts. Today, humanitarian and peacekeeping operations place our troops in regions around the globe, and expose them to endemic pathogens to which their immune systems are naive. Soldier health and performance can be compromised by a variety of diseases for which there are no effective preventive or therapeutic countermeasures. Research toward the development of effective pretreatments, vaccines, and therapies requires the use of specific animal models in assessing safety and efficacy.

During Operations Desert Storm in the Persian Gulf and Restore Hope in Somalia, outbreaks of respiratory diseases, diarrheal diseases such as shigellosis, and parasitic diseases such as *leishmaniasis* and malaria threatened the health and well-being of our troops. Indeed, the DoD is still assessing and addressing concerns over the long-term effects of various environmental, physical, and medical factors associated with the Persian Gulf Conflict. It is obvious that the health and well-being of military personnel extend far beyond the immediate scope of the battlefield.

One of the most critical areas requiring DoD animal use is the compelling need to develop vaccines, drugs, and therapies to protect, sustain, and treat service men and women during military operations. These therapeutics are needed for protection against numerous militarily relevant diseases and threats, many of which can result in potentially fatal diseases or conditions that have no known treatments, therapies, or cures. Ethical responsibilities, as well as regulatory requirements of the Food and Drug Administration (FDA), necessitate that candidate vaccines, drugs, and devices be demonstrated safe and efficacious in laboratory animal models prior to initiation of human use protocols whenever possible. Drug efficacy screens are generally conducted at the lowest possible phylogenetic level (i.e., in rodents). Given that drug response is often species-specific, promising drugs are subsequently tested in nonhuman primates (NHP). During the final stages of vaccine and drug development, large-scale safety and efficacy testing is usually conducted using human volunteers.

The DoD must develop the materiel and technological means to provide critical and immediate battlefield injury care to service men and women. This is often provided by field medical personnel in an austere, harsh, and hostile environment, hours away from full hospital medical care. This contrasts markedly with medical facility counterparts in the civilian community that generally possess well-appointed emergency medicine and trauma management systems. A domestic, low velocity projectile gunshot patient in a modern civilian shock and trauma center will be supported and managed by a full complement of medical, surgical staff, and a full complement of pharmaceutical supplies in their armamentarium. The combat casualty may be supported by only a single field medic or buddy and the medical supplies, experience, and expertise he or she can carry. No in vitro model can simulate the range of effects of multiple organ failure or shock that so often follows physical trauma.

There are numerous research areas, including medical chemical and biological warfare defense, where animal-based studies are particularly critical because human use protocols are simply not possible in the search for understanding and developing protection against many highly lethal agents. Ethical considerations severely restrict or preclude the use of clinical studies in this research area. The world is no longer a place where the deadly chemical poisons and pathogens of mass destruction are controlled by the infrastructure of national governments. Terrorist organizations have demonstrated a ruthless disregard for human life, fomenting mass murder on a previously unimaginable scale. Rogue nations, some with weapons of mass destruction, are in a

position to transfer these destructive technologies to organizations seeking to attack U.S. civilians and military personnel. Terrorists have already released both chemical and biological weapons in Japan, and U.S. civilians have been targeted with anthrax. The sheer magnitude of these threats underscores the need to develop protective medical countermeasures for both military and civilian personnel. The DoD is charged with the responsibility of identifying and developing these defensive countermeasures to protect the nation, and carefully regulated animal studies are critical to the success of biomedical research programs supporting, for example, the development of safe and effective vaccines for anthrax and smallpox.

This responsibility of the DoD to maintain the health of men and women and their families wherever they work, on military installations, on the battlefield, or in peacekeeping missions around the world, underlies the need for the DoD to conduct research and to train and educate military health care providers. Clinical investigation programs at Medical Treatment Facilities support postdoctoral Graduate Medical Education (GME) programs, in which physicians receive residency training in special areas such as orthopedics, surgery, and emergency critical care. To be certified, the GME programs must demonstrate that a medical facility has programs to provide research opportunities for both staff and students. These clinical investigation programs provide training in the performance of research involving both laboratory animals and human subjects. This combined capability increases the opportunities for staff and GME students and significantly enhances their training, thus enabling the warfighter to receive the best care possible. This capability also increases the opportunities for patients who desire to participate in research protocols such as the Multicenter Oncology and Pediatric Oncology protocols. In this regard, Congress has mandated that the DoD will work closely with the National Institutes of Health (NIH) to provide more opportunities for DoD beneficiaries to participate in the NIH-sponsored protocols. Many of the clinical investigation training protocols, such as surgical skills training for microvascular or reproductive surgery, support GME programs that follow requirements set by the American College of Surgeons. These courses provide essential opportunities for the training of medical personnel who will work in both military and civilian sectors. Programs using animals for GME training are also subjected to veterinarian oversight, and these animals are maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC).

The use of animals is also important in the DoD's nonmedical programs. These studies include the development of biological sensors, sonar, echolocation, biorobotics, aviation construction materials, and hearing and eye protection systems. There are also nonmedical studies to understand learning and memory physiology in an attempt to model the brain's circuitry for advanced data processing computers and robotic machinery. These advanced computers and robots will eventually reduce the risk that our service men and women encounter in their daily duties. The DoD performs marine biology research to better understand the military working marine mammals. In addition, the marine mammals are investigated to determine their auditory detection thresholds in marine use as sentries. Studies of biosonar systems are conducted to enhance the use of military marine mammal systems for mine detection and retrieval, personnel detection, and reconnaissance.

## **I.3 BENEFITS OF ANIMAL RESEARCH**

The DoD laboratories and extramural contractors provide the capability needed to address the medical and nonmedical challenges of the future through the efforts of internationally renowned medical and scientific experts working in state-of-the-art facilities and in the field. The Department conducts and resources RDT&E and training missions to sustain the operational capabilities of today's service men and women. Many of these programs require the use of animals to meet mission requirements and result in benefits for both the military and the civilian sector (Tables I-1, I-2, and I-3). The military benefits from supporting research programs in areas that currently threaten military personnel, such as combat trauma, chemical and biological agents, infectious diseases not endemic to the United States, directed energy, and occupationally unique health hazards from military operations and environmental extremes. These research programs contribute significantly to the readiness and sustainment of the DoD's warfighting capability and focus heavily on the prevention of casualties. These benefits reflect the diversity of the DoD research efforts in support of joint warfighter needs ([Benefits of DoD Intramural RDT&E and Training](#)).



It is important to recognize that DoD research requirements ~~also~~ benefit civilians both in the United States and in the world community. The DoD indirectly or directly advances understanding of our knowledge of cardiovascular disease, trauma care and treatment, respiratory injuries, burns, and specific surgical procedures. The DoD's role in some of these areas is critical in that some of these areas traditionally receive only modest funding support in civilian research programs. Marine researchers and policymakers also benefit from DoD marine mammal research through its indirect contribution of a better understanding of the impact on marine mammals of noise pollution from ships.

With the end of the Cold War, Congress has tasked the DoD to manage medical research that directly benefits the civilian population such as research in breast, prostate, and ovarian cancer. These model research programs, developed with guidance from the National Academy of Sciences, account for a considerable portion of DoD extramural animal research and are having an immense and positive impact on the understanding, prevention, and treatment of these and other diseases. Transgenic mice, for example, are critical for determining gene effects on the development and progression of cancers. No in vitro system exists that can model the extremely complex cellular and molecular "crosstalk" between tissues and cells.

The infectious disease and medical chemical and biological defense research programs are primarily designed to develop countermeasures to potential threats to U.S. military personnel who must operate in a global setting. In FY01, these research programs were awarded patents as shown in Table I-2. While the underlying requirement for disease research is to protect U.S. military men and women, it should be noted that there is an indirect benefit of DoD's research to the broader world community. The scant resources of many poorer nations are directed at basic survival needs such as food and medicine, and not research. Because U.S. troops must operate in a worldwide theater, the DoD has had a long-standing commitment to the development of countermeasures against malaria, the disease that annually kills more people than any other. In addition, there are many examples of direct humanitarian benefits of the DoD investment and collaborative efforts with other nations to improve the quality of life of both humans and animals. Several prime examples of the humanitarian benefits of DoD research efforts are noted in Table I-3.

Besides the medical benefits of animal research, there are many nonmedical and training benefits. The development of biosensors and the identification of environmental hazards benefit military and civilian communities alike. The DoD has many exceptional medical and scientific educational programs that train both medical personnel and scientists. While these people are in the military, the DoD reaps the benefit of this training; once they leave the military and apply their training in the private sector, the civilian community realizes this benefit. The DoD's development of alternatives to reduce or replace animals provides an extra value to both communities and to animals. Also, refinement research results in more humane methods of performing research that is applied in many types of research settings.

In FY01, the DoD reported nearly 450 publications in scientific journals, proceedings, technical reports, books, and book sections from RDT&E efforts that required the use of animals.



**Table I-1** Animal Use Benefits**Clinical Investigations**

- Clinical research on the development and testing of HIV vaccines
- Better understanding of the development, diagnosis, and treatment of colon carcinomas
- Identification of induced antibody responses to vaccine development
- Treatment and prevention of hemorrhagic shock
- Treatment of acute lung injury
- Treatment of advanced prostate cancer
- Treatment and prevention strategies for neuropsychiatric disorders
- Determination of active mechanism affecting altered fluid handling in alcohol exposure
- Research on skin transplants

**Medical**

- Development and evaluation of malaria vaccines
- Antigen detection during vaccine development
- Development of meningococcal and anthrax vaccines
- Identify mechanism of dermal tissue damage during lesion development
- Determine methodology for inducing controlled hypothermia
- Evaluation of the acute effects of laser exposure
- Research on the mechanisms of occupational and chronic fatigue
- Quantification of munitions compound toxicity on wildlife
- Research on the development of lymphoma
- Researching blast overpressure exposures
- Development of active topical skin protectants against chemical warfare agent exposure
- Determination of the molecular mechanisms, detection, and treatment of breast, prostate, and ovarian cancers, and neurofibromatosis

**Nonmedical**

- Updating of the national and international laser safety standards
- Identification of environmental and human health risks factors
- Developing methods and technologies for toxicity testing
- Developing preventative measures for environmental toxins
- Developing biomonitoring systems
- Evaluating toxicological hazards of occupational chemical exposure

**Training**

- Graduate medical education training
- Training of surgical residents in a variety of critical skills
- Advanced trauma life support and medical emergency training
- Veterinary personnel medical emergency training
- Training for research and animal care personnel to improve handling techniques and protocol procedure performance

**Table I-2** Patents Resulting from Animal Use Research in FY01

- A vaccine for dengue virus
- A microsphere delivery system for the controlled release of antiinflammatory drugs
- A diagnostic method for detecting *Cyclospora*, a water-borne pathogen and public health concern
- Protein peptides that will serve as candidate compounds toward developing an HIV vaccine
- A topically applied skin cream that blocks and inactivates chemical warfare agents
- A compound that helps protect against the effects of head injury
- A noninvasive pulse oximeter for measuring blood oxygenation

**Table I-3** Humanitarian Benefits of DoD Research Efforts

In Peru, the DoD has investigated epidemiology of viral hemorrhagic and encephalitic diseases among civilians and deployed military troops in Peru. This research has demonstrated that the arthropod-borne viruses most commonly associated with human disease in the Amazon region were dengue, Oropouche, and Venezuelan equine encephalitis (VEE). In addition, Yellow Fever, Mayaro, VEE virus, and one case by an apparently new Phlebovirus (family Bunyaviridae) were isolated from febrile patients in an outbreak in the high jungle near Cusco, Peru. This was the first isolation of Maguari virus, which is associated with human disease.

The DoD performs critical diagnostic analyses of suspected disease outbreaks in the United States and overseas and provides vaccine materials for both humans and animals in emergency settings. DoD research facilities were at the forefront of efforts to diagnose and control outbreaks of: (1) deadly hantavirus infection among Navajo Native Americans in 1993; (2) Rift Valley fever in Egypt in 1993; (3) VEE virus in people and horses in central and South America in 1995; (4) Ebola and related viruses in Zaire in 1995; (5) West Nile virus in New York citizens, horses, and birds in 1999; and (5) anthrax distributed by mail in 2001.

Malaria is one the world's greatest killers, and the DoD's fielding of new drugs is critical in the face of the development of resistance to currently fielded drugs. The Army antimalarial researchers have tested over 500,000 drugs and other substances for activity against malarial pathogens. One new drug developed by the Army, tafenoquine, is highly effective in both malaria prevention and therapy. Should tafenoquine prove safe and efficacious in remaining studies and become licensed by the FDA, it would provide a significant addition to our ability to protect deployed military personnel and civilian populations against malaria.

The DoD collaborated with the Argentine government in the development of the Junin vaccine that has provided critical, 98% effective protection for more than 120,000 individuals in endemic areas of Argentina against the ravages of Argentinean hemorrhagic fever.

## I.4 SCOPE OF REPORT

This report covers DoD animal research in the context of education, training, and RDT&E both in DoD laboratories and by extramural projects funded by the Department for FY01. The two major components of the FY01 report are: (1) a summary of animal use with regard to species, DoD components, research area and USDA pain category (Section II), and (2) DoD initiatives to promote alternative methods that replace, reduce, or refine animal use (Section III). This report does not include information on animals used by the DoD solely for the purpose of food preparation for human or animal consumption, ceremonial activities, recreation, or the training, care, and use of military working animals. Information was solicited and received from DoD Military Commands, Agencies, and Activities and from non-DoD organizations involved in DoD-supported animal care and use programs. For the purpose of this report, an intramural program represents research performed at a DoD facility and funded by either DoD or non-DoD funds, while an extramural program represents research performed

by a contractor or grantee that is funded by the DoD. In FY01, data were acquired from all 1,184 extramural activities (civilian institutions conducting DoD-funded research) and 35 DoD organizations.

Additional information regarding the DoD Animal Care and Use Program can be found at <http://www.dtic.mil/biosys>. Policies, the standard research protocol format, the Biomedical Research Database (containing descriptive summary information of current DoD animal research projects), and prior year annual reports are provided at this web site.

#### *1.4.1 DoD Animal Use Profiles by Research Category*

A profile of animal use in the DoD is provided in Section II. The DoD utilizes a system for classifying all animal use that is broken down into 7 primary categories and 20 subcategories (see Table II-1). It should be noted that no animals in any of these areas were reported as used for development or testing of offensive weapons. Detailed charts and graphs are included in Section II.

In FY01, the DoD used 330,149 animals, which is a 9% decrease from FY00 and a 45% decrease (270,534) from FY94. Of the FY01 group, only 21,643 (7%) were USDA reportable species as defined in the AWA. This year 169,156 animals were reported used in intramural research programs and 161,993 were used in extramural programs. The use of intramural animals decreased nearly 10% (18,078) in FY01 compared with FY00 use and decreased by 37% (98,935) compared with FY94 use. The number of animals used in extramural research was 10% (17,599) less in FY01 than the number in FY00 and 52% less (171,576) than the number used in FY94. Table I-4 summarizes the breakdown of FY01 animal use by species and animal use category.

**Table I-4** Summary of DoD Animal Use Statistics

Total Animal Use by Species	% of Total	Total Animal Use by Category	% of Total
Fish, amphibians, reptiles, and birds	7.3	Medical	84.2
Rodents	89.0	Nonmedical	5.4
Rabbits	0.9	Clinical Investigation	3.9
Farm Animals (i.e., sheep, pigs, cows, horses and goats)	1.6	Training and Instructional	1.5
Dogs, cats, nonhuman primates, and marine mammals	0.7	Adjuncts/Alternatives	4.8
Other	0.5	Classified Secret or Above Studies	0.01
		Other Animal Use	0.2

#### *1.4.2 DoD Initiatives to Promote Alternative Methods that Replace, Reduce, and Refine the Use of Animals*

Congress requested that federal departments and agencies establish aggressive programs to replace, reduce, and refine current use of animals. A review of DoD programs and initiatives to develop and implement alternatives to animal research is reviewed in Section III.

Animal research is an essential part of the scientific process, but it is only initiated after due consideration of alternatives. The DoD uses a [Standard Protocol Format \(SPF\)](#) that specifically requires each investigator to consider alternatives to the use of animals and to justify the animal model selected. The IACUC animal use oversight process includes a strong emphasis on consideration of alternatives in all protocols. All protocols that involve relieved or unrelieved pain or distress require consultation with a veterinarian prior to IACUC review and a database search for scientifically acceptable alternatives to the proposed method. Each protocol that involves animals in research or training must explain the need for animal use and defend the choice of species as the most scientifically valid model. Often, economies of time and resources are gained when scientifically valid alternatives to animal use are available. Scientists in the DoD have developed and adopted many alternatives to animal use.

Alternatives are addressed in the report as being either general or specific. General alternatives are those that are frequently implemented in many different DoD programs. Specific alternatives are those that may be specific to a research protocol and/or facility. Alternatives presented in Section III are both those developed by DoD investigators and those implemented during FY01. Replacement includes the elimination of animal use altogether, generally by adopting in vitro or theoretical model study systems. Replacement also includes the substitution of species that are higher on the phylogenetic scale with those that are lower. Reduction is the use of fewer animals without loss of scientific test validity. Refinements include changes in methods that reduce or eliminate animal distress or pain or improve animal quality of life while maintaining or improving the quality/quantity of research data collected.

In FY01, over 770 animal use projects reported that they were implementing alternative methods in animal use. Table I-5 presents examples of alternatives developed by the Department in FY01 to replace, reduce, and refine the use of animals. In addition, the Department sponsors conferences and workshops to promote alternatives to animal research.

**Table I-5** *Examples of Alternatives for Replacement, Reduction, and Refinement of the Animal Developed or Being Developed by the DoD in FY01*

- In an assessment of toxicity on reproductive organ development, frogs have replaced rodents.
- A noninvasive, tail-cuff method to measure blood pressure has replaced an invasive method in rats.
- Membrane feeding has reduced the number of animals necessary to maintain mosquito colonies.
- Telemetry is being used to monitor physiological parameters, reducing stress in a number of monkey and rat studies.
- A snake model is being developed to replace the rhesus monkey in retinal research, employing noninvasive procedures.
- Virtual models and robotic trauma manikins have replaced the use of a substantial number of goats in Advanced Trauma Life Support Courses.
- Procedures and methods for improved environmental enrichment continue to be developed and evaluated for many research animal species.

## I.5 CONCLUSION

It remains essential to use animals in DoD RDT&E and training to protect the health and lives of military personnel. Although alternatives to animal use will continue to be vigorously sought and applied as possible, the complex interactions of organ, tissue, cell, disease agents or processes, and environment make the continued judicious use of animals in DoD programs necessary. Animals are used in research only when scientifically acceptable alternatives are not available. The DoD is committed to full ethical responsibility and regulatory compliance for its animal-based research programs. The Department's animal care and use requirements are as strict or stricter than those required of non-DoD, government-funded, or public and private research institutions. The DoD policy directs all facilities maintaining animals for use in research and training to apply for AAALAC accreditation, and the DoD has established effective programs to replace, reduce, and refine current use of animals.

## SECTION II

# DoD ANIMAL USE PROFILES

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The information presented in this section provides profiles on the reported use of animals by component, species, animal use category, and the USDA pain categories.

### II.1 METHODS

Information was solicited and received from DoD components and DoD-funded organizations involved in animal care and use programs located both in and out of the United States for fiscal year 2001 (FY01). These included extramural contractors and grantees that performed animal-based research. For the purpose of this reporting requirement, an intramural program represents research performed at a DoD facility and funded by either DoD or non-DoD funds. An extramural program represents research performed by a contractor or grantee that is funded by the DoD.

#### *II.1.1 Animal Use Profiles*

The animal use profiles prepared for this report are consistent with the reporting information and data provided to the USDA Animal and Plant Health Inspection Service (APHIS) Form 7023. In addition, this report contains comprehensive information on all other animals (e.g., mice, rats, and birds) used that are not required to be reported to the USDA.

For the purposes of the FY01 reporting requirement, an animal was defined as any live nonhuman vertebrate used for RDT&E and training. Only live animals that were either on hand in the facility or acquired and used during FY01 are included. Carcasses, animal organs, tissues, cells, blood, fluid components, and/or by-products purchased or acquired as such animal/biological components are not reported. This report does not include animals used or intended for use as food for consumption by humans or animals, animals used for ceremonial purposes, or military working animals and their training programs.

A single animal was counted only once in determining the number of animals used during the fiscal year for a particular work unit or protocol. Breeding animals or animals on hand during FY01 but not actually used during the fiscal year are not included in the numbers reported here.

#### *II.1.2 Animal Use Categories*

Military departments and extramural organizations involved in the performance and/or funding of animal care and use programs reported animal work by the category that best describes the general research or training purpose of the animal use. The 7 primary categories and 20 subcategories are listed in Table II-1. If the [Animal Use Categories](#) provided did not adequately describe the animal use within each particular work effort, the animal was placed in the Other Animal Use Categories.

**Table II-1** Animal Use Primary Categories and Subcategories

<p><b>MEDICAL (M)</b></p> <p>M1: Military Dentistry  M2: Infectious Diseases  M3: Medical Chemical Defense  M4: Medical Biological Defense  M5: Human Systems Technology  M6: Combat Casualty Care  M7: Ionizing Radiation  M8: Other Medical RDT&amp;E</p> <p><b>NONMEDICAL (N)</b></p> <p>N1: Physical Protection  N2: Physical Detection  N3: Offensive Weapons Testing  N4: Other Nonmedical RDT&amp;E</p> <p><b>CLINICAL INVESTIGATIONS (C)</b></p> <p>C1: Clinical Medicine  C2: Clinical Surgery  C3: Other Clinical Investigations</p>	<p><b>TRAINING/INSTRUCTIONAL (T)</b></p> <p>T1: Training, Education, and/or Instruction of Personnel  T2: Other Training/Instruction</p> <p><b>ADJUNCTS/ALTERNATIVES TO ANIMAL STUDIES (A)</b></p> <p>A1: Adjuncts to Animal Use Research  A2: Alternatives to Animal Investigation  A3: Other Alternatives/Adjuncts</p> <p><b>CLASSIFIED SECRET OR ABOVE STUDIES (S)</b></p> <p>Studies classified secret or above</p> <p><b>OTHER ANIMAL USE CATEGORIES (O)</b></p> <p>Other animal use purposes</p>
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### II.1.3 USDA Pain Categories

The USDA requires that all institutions using regulated animals for research, testing, training, or experimentation register with the USDA as a research facility and submit an annual report. In that report, animals are assigned to one of the three USDA pain/distress categories (Table II-2). As noted above, this report includes animal species not regulated by the AWA and its implementing regulations.

**Table II-2** USDA Pain Categories (USDA APHIS Form 7023)

<p><b>USDA COLUMN C</b></p> <p>Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.</p> <p><b>USDA COLUMN D</b></p> <p>Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.</p> <p><b>USDA COLUMN E</b></p> <p>Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.</p>
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The animals reported in Column C of the USDA report are those used in a procedure that would reasonably be expected to cause not more than slight or momentary pain and/or distress in a human being to which that procedure was applied. Procedures performed on these animals are those that are usually conducted on humans without anesthesia or analgesia. Examples include most blood-sampling techniques (excluding intracardiac blood sampling), injections, and tattooing.

The animals reported in Column D of the USDA report are those in which pain is alleviated or controlled by appropriate anesthetic, analgesic, or tranquilizing drugs. Examples include anesthesia for surgical procedures or catheter placement, and analgesia during recovery from surgery.



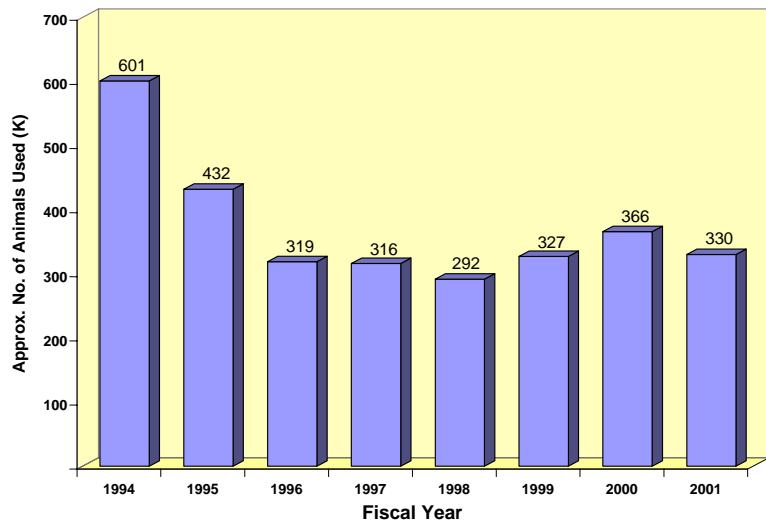
The animals reported in Column E of the USDA report are those that experience more than slight or momentary pain or distress because the administration of pain-relieving drugs would adversely affect the study. Examples of procedures where drugs were not used because they would have adversely affected the procedures, results, or interpretation of the research, or tests include some infectious disease studies and some toxicology studies.

All procedures that involve animals reported under USDA Pain Category Columns D or E are extensively reviewed during the protocol approval process. Prior to formal protocol review, a veterinarian with experience and/or training in laboratory animal medicine (LAM) must review all procedures. In addition, the primary investigator must write a justification for all procedures for animals reported under Column E. The DoD standard protocol states, "Procedures causing more than transient or slight pain that are unalleviated must be justified on a scientific basis in writing by the primary investigator. The pain must continue for only the necessary period of time dictated by the experiment, and then be alleviated, or the animal humanely euthanized." Moreover, the primary investigator must sign an assurance statement that alternative procedures are not available, and the IACUC must review and approve all procedures before the study begins.

## II.2 RESULTS/DISCUSSION

### II.2.1 General Results

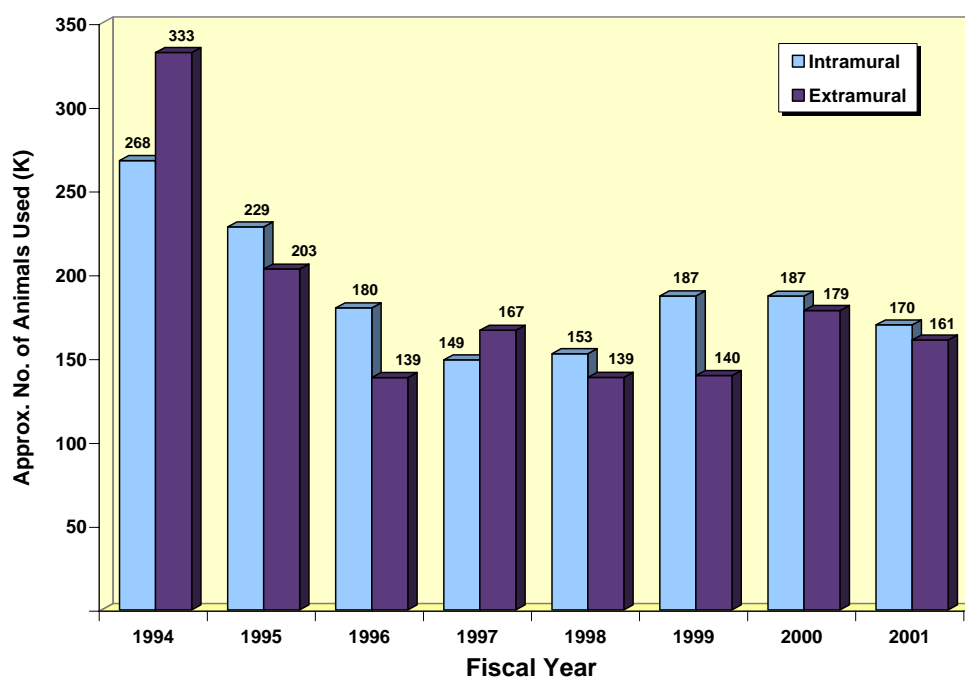
There was a total of 330,149 animals reported used in FY01, which is over a 9% decrease from FY00 and a 45% (270,534) decrease from FY94 (Figure II-1). It should be noted that these numbers include rats, mice, birds, frogs, and fish. None of these animals are required to be reported under the AWA. Using the definition of *animal* under the AWA, the DoD would report a much lower total of 21,643 animals for FY01, which is less than 7% of the total of all animal actually used, regardless of species. DoD's non-restrictive definition of *animal* reflects a much higher level of accountability.



**Figure II-1** DoD Animal Use by Year

In FY01, 169,156 animals were reported used in intramural research programs and 161,993 were used in extramural grants or contracts (Figure II-2). FY01 intramural animal use decreased nearly 10% (18,078) compared with FY00 use and decreased 37% (98,935) compared with FY94 use. The number of animals reported used in extramural research was 10% (18,079) less in FY01 than the number in FY00 and 52% (171,599) less than the number used in FY94. Given that the level of funding for extramural programs varies from year to year, depending on Congressional funding and DoD priorities, the total number of extramural projects employing animals fluctuates with changes in the number of contracts and grants awarded. Furthermore, many extramural research projects use animals only during a portion of the proposed project (e.g., third year of project) while others use animals throughout the entire project.





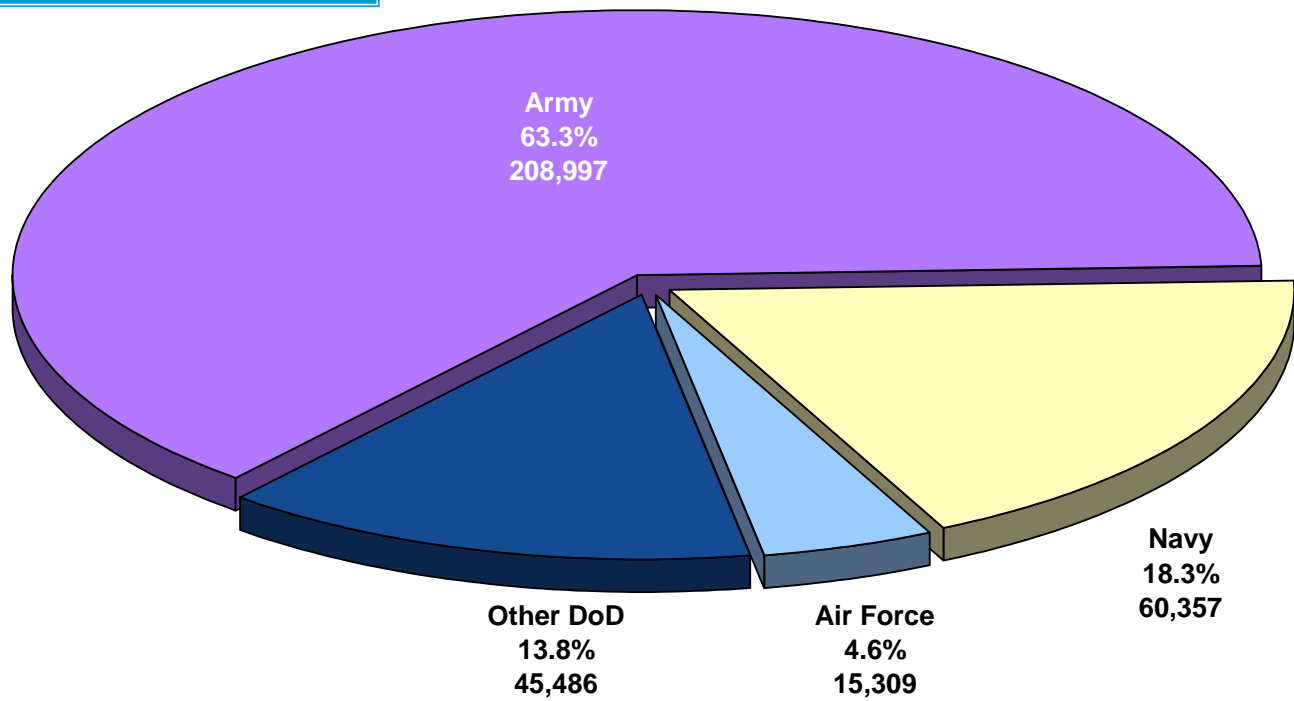
**Figure II-2** Intramural/Extramural Animal Use by Year

Since 1994, there has been a remarkable decrease in animal use in intramural and extramural activities that directly support DoD mission requirements. Congressionally Directed Medical Research Programs (CDMRP), which are directed by Congress to be overseen by the US Army and fund areas such as prostate and breast cancer research, do not address DoD mission requirements. These programs fund extramural activities that comprise nearly all of DoD animal use category M8 (Other Medical RDT&E) and used over 111,000 animals (34% of all DoD animals used in FY01). Before 1995, very little non DoD-mission required research was conducted. This changed dramatically with the first large congressional appropriation of approximately \$225 million in FY95. When considering only DoD-mission required research there is a large decline in extramural animal use since FY94 exceeding 200,000 animals

## II.2.2 Animal Use by Military Department

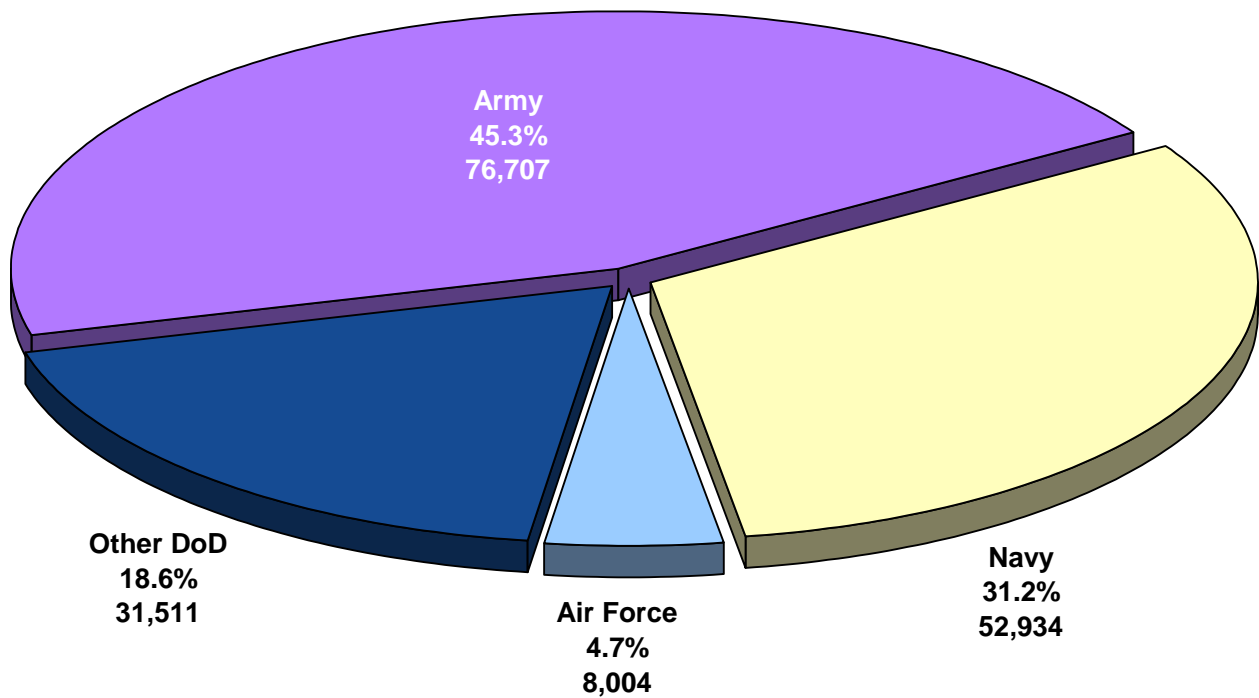
Information concerning total reported DoD use of animals by each military department is presented in Figure II-3. Figures II-4 and II-5 show the intramural and extramural animal use by department, respectively.

***TOTAL = 330,149***



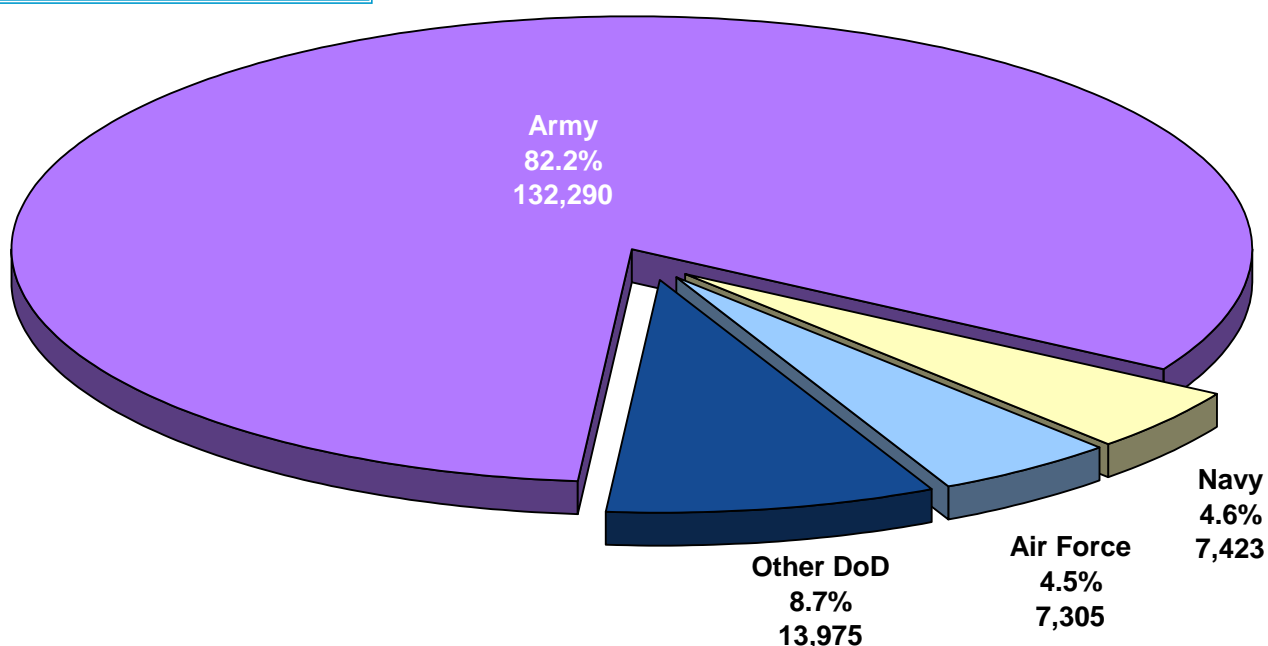
**Figure II-3** DoD Intramural and Extramural Animal Use by Military Department for FY01

***TOTAL = 169,156***



**Figure II-4** DoD Intramural Animal Use by Military Department for FY01

**TOTAL = 160,993**



**Figure II-5** DoD Extramural Animal Use by Military Department for FY01

In FY01, the Army used 63% of the total number of animals reported used by the DoD, 45% of the total intramural animals, and 82% of the total extramural animals. Overall the Army's animal use decreased by 16% between FY00 and FY01. The majority of this decrease (67%) was in the intramural programs where there was a 26% drop (-26,-998) in the Army's reported animal use. There was also a 9% (13,012) decrease in the Army's extramural animal use since FY00.

Table II-3 shows the complete list of congressionally directed (CDMRP) research programs, all of which are managed by the Army with funding dependent on yearly congressional appropriations. These programs used the majority (84% or 111,286) of the Army's extramural research animals and 34% of the total DoD animal use in FY01. Among all of the Army's extramural programs, the Breast Cancer Research Program utilized the largest number of animals (57,382) although this value declined from FY00 levels by 3,782 in FY01. This program alone accounts for 17% of all FY01 animals used by the DoD. Among the other larger extramural research programs, numbers rose slightly for the Prostate Cancer Research (1,748), Neurotoxin Research (2,290) Programs, and declined for the Bone Health/ Osteoporosis Research Programs (-4,660).

The U.S. Army is also the congressionally mandated lead agency for infectious disease and military dentistry research and the DoD Executive Agency for medical chemical and biological defense and nutrition studies. The U.S. Army research on infectious diseases and on chemical and biological defense employed 23,348 and 44,301 animals, respectively, showing 10% and 39% declines in animal use in these areas from FY00.

**Table II-3** U.S. Army - FY01 Congressionally Directed Medical Research Programs

Army's Congressionally Directed Medical Research Programs (\$M)	
Alcoholism Research	8.5
Breast Cancer Research	175.0
Cancer Research Centers	10.5
Lung Cancer Research	4.5
Militarily Relevant Disease Management	7.1
Neurofibromatosis Research	17.0
Ovarian Cancer Research	12.0
Prostate Cancer Research	100.0
Prostate Disease Research	7.5
Peer Reviewed Research Program <sup>1</sup>	50.0

<sup>1</sup>The Peer Reviewed Research Program addresses a variety of topics including: acute lung injury, asthma, militarily-relevant disease management, biohazard detection, arthropod transmitted infectious disease, Gulf War illness, obesity, sleep management, alcohol abuse, laser eye injury, smoking cessation, medical technologies

Relative to FY94, the Army has decreased its intramural use of animals by 54% (-89,953) and its extramural use by 55% (-159,973). Overall the Army has decreased its use of animals in research by 54% since FY94. As pointed out earlier (Section II.2.1), the bulk of animal use under Army-administered programs derives from non-DoD mission-required activities directed by Congress. Discounting the considerable volume of CDMRP research, Army mission-related animal use has fallen by nearly 80% since FY94, when over 450,000 animals were used by that service.

The Navy used 18% of the total number of animals reported used by the DoD, 31% of the intramural animals, and 5% of extramural animals. Comparing animal use in FY01 to FY00, there was a 27% increase in the total number of animals. This increase is primarily attributable to an additional 12,138 animals used in intramural research projects. This represents an increase of 30% in Navy intramural animals, but is still 14% lower than the Navy's intramural total from FY99 when the Global Emerging Infections Surveillance and Response System (GEIS) program was implemented. The GEIS program was the result of Presidential Decision Directive NSTC-7 in June 1996 directing all federal agencies to cooperate in surveillance and research on new infectious disease problems. Because of its wide-ranging assets for disease control, the mission of the DoD was expanded to support global surveillance, training, research, and response to emerging infectious diseases. Navy extramural animal use was only slightly increased from FY00 (855).

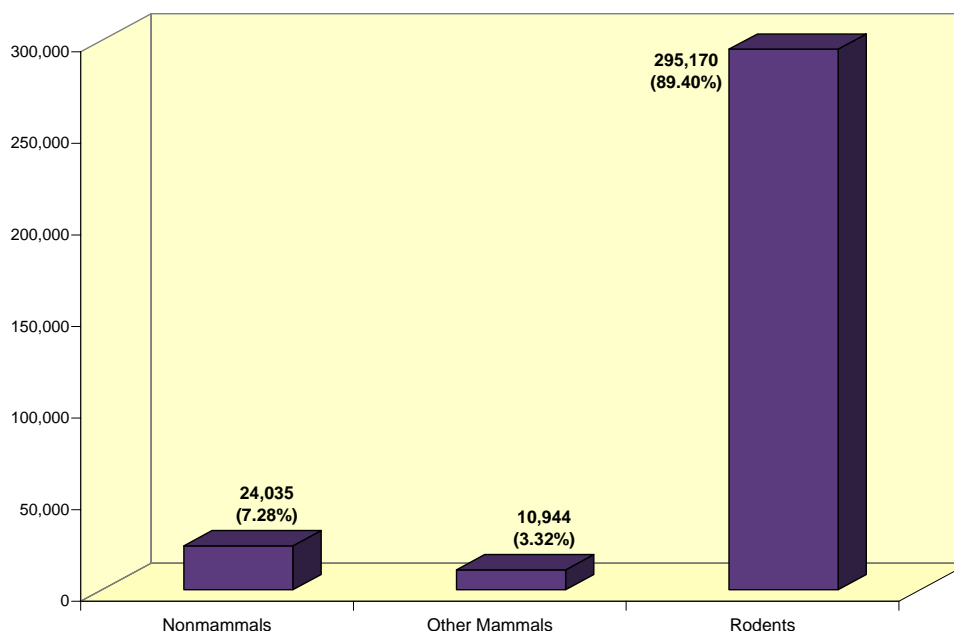
The majority of animals (93%) used by the Navy in FY01 were used in medical research. Broken down further, infectious disease and combat casualty care research accounted for 81% and 11% of the Navy's animal use, respectively. Naval animal use is about 35% (19,226) higher than that in 1994. The Navy conducts considerable infectious disease research on the pathogenesis of, and development of medical countermeasures to, dengue viral illness and malaria, two mosquito-borne diseases.

Within the DoD, the Air Force used the fewest number of animals. In FY01 it used 5% of the total number of animals reported used by the DoD, 5% of the intramural animals, and 5% of the extramural animals. Compared to FY00, Air Force intramural animal use increased by 904, while extramural animal use increased by 4,652 resulting in an overall increase of 5,556. The Air Force used 82% and 10% of its animals in nonmedical research and clinical investigation projects, respectively. Relative to FY94, the Air Force has shown a 60% decrease (-22,669) in the use of research animals.

The remaining group, "Other DoD", includes the Uniformed Services University of the Health Sciences (USUHS), Defense Advanced Research Projects Agency (DARPA), Armed Forces Radiobiology Research Institute, and the Armed Forces Institute of Pathology. These OSD components used 14% of the DoD total animals, 19% of the total intramural animals, and 9% of total extramural animals. There was a 23% (13,465) decrease in animal use by the OSD components in FY01 relative to FY00. This decrease was seen in both the intramural (-4,094) and extramural (-10,071) programs. Overall the OSD components used the majority (89%) of their animals in clinical investigations (7,519) and medical research (35,740). The majority of clinical research animal use was in clinical medicine projects (6,908). Biological defense (12,021) and ionizing radiation research programs (14,135) both declined from FY00 by 55% and 27%, respectively. Compared to FY94 there has been a 23% (13,495) decrease in the OSD component animal use.

### *II.2.3 Animal Use by Species*

The DoD uses three major classifications for reporting vertebrate animal use: rodents, nonmammals, and other mammals. Rodents are the primary type of animals used in each animal use category and accounted for 89% of the DoD's animal use in FY01. This year the use of rodents decreased by 37,669 (Figure II-6), a 11% increase due to a 13% (35,193) reduction in the use of mice. Compared to FY00, there was a 26% decrease (-843) in the use of hamsters and a 6% decrease in the use of guinea pigs (-418). Other rodent species are represented in much smaller numbers. Compared with FY00, in FY01 there was a 15% (3,119) increase in the reported use of non-mammals and a 9% (1,104) decrease in the number of other mammals used. The vast majority (97%) (319,205) of animals used by the DoD in FY01 were rodents, birds, amphibians, reptiles, and fish.



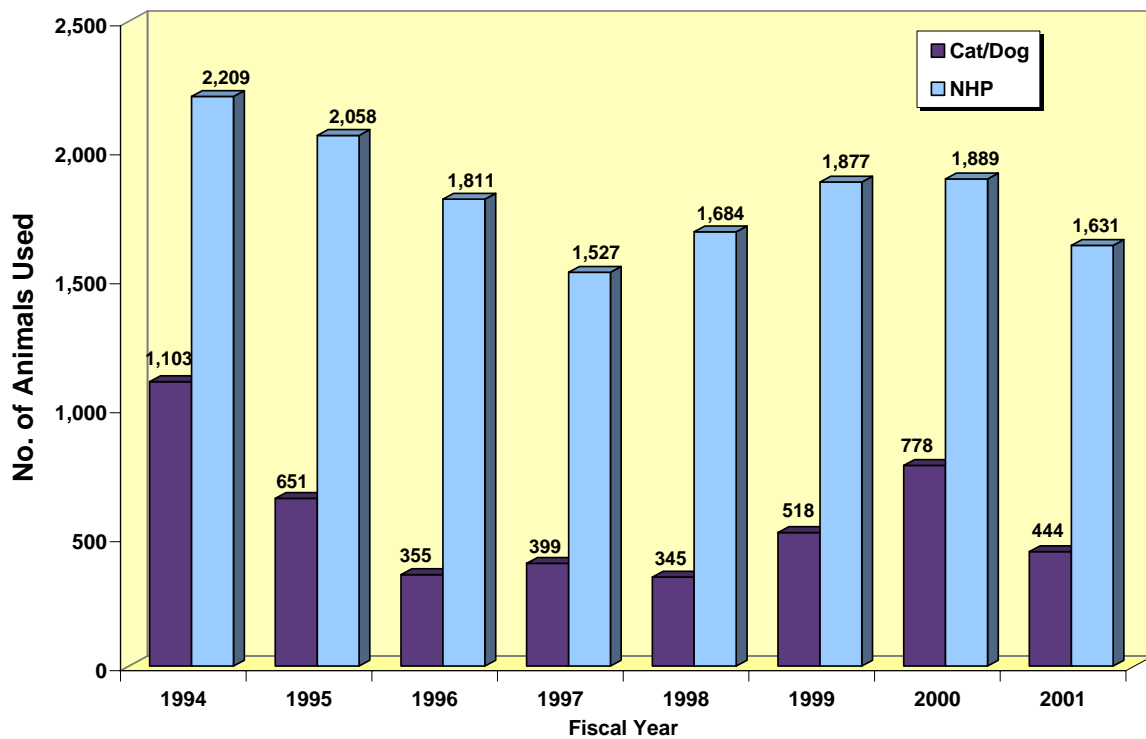
**Figure II-6** Nonmammals, Other Mammals, and Rodents for FY01

Since FY94, there have been significant decreases in the number of species used by the DoD. There has been an 83% (114,757) decrease in nonmammals, a 34% (152,692) decrease in rodents, and a 21% (2,877) decrease in other mammals. Animals used in FY94 but not used in FY01 are shown in Table II-4. There have been significant decreases in the use of large animals such as marine mammals, horses, sheep, and goats. For example, between FY94 and FY01, there was a 56% (62) decrease in marine mammals and many species were not used in FY01 (Table II-4). There were also decreases in the use of horses (-78), goats (-1,452), sheep (-214), bats (-72), and degus (-12). At the same time, there was an increased use of swine (889), chinchillas (99), ferrets (96), and reptiles (42). Overall, there has been a shift from the use of large animals to smaller animals and a shift to those that are lower on the phylogenetic scale.

**Table II-4** Vertebrates Used by the DoD in FY94 and not in FY01

Nonmammals	Other Mammals
<i>Amphibian</i>	Armadillo
African Toad	Burro
Salamander	Civet
	Fox
<i>Avian</i>	Opossum
Duck	Owl Monkey
Falcon	
Pigeon	<i>Marine Mammals</i>
Robin	Beluga Whale
Sparrow	Bottlenose Dolphin
Starling	Commerson's Dolphin
<i>Reptiles</i>	Elephant Seal
Chameleon	False Killer Whale
Monitor Lizard	Fin Whale
	Gray Whale
<b>Mammals</b>	Harbor Seal
<i>Rodents</i>	Pilot Whale
Prairie Dog	Risso's Dolphin
	White-Sided Dolphin

In FY01, there was a decrease in the combined use of NHPs, dogs, and cats relative to FY00. When comparing FY00 to FY01, there was a decrease in the use of NHPs (258), dogs (284), and cats (50) (Figure II-7). NHPs were primarily used in medical research (72%), clinical research (11%) and training (10%). Within medical research, they were primarily used in infectious diseases (59%) and biological/chemical defense (28%). NHPs are unique in their ability to model human response to therapeutic compounds and are used in advanced preclinical research.



**Figure II-7** Use of Nonhuman Primates and Dogs and Cats by Year

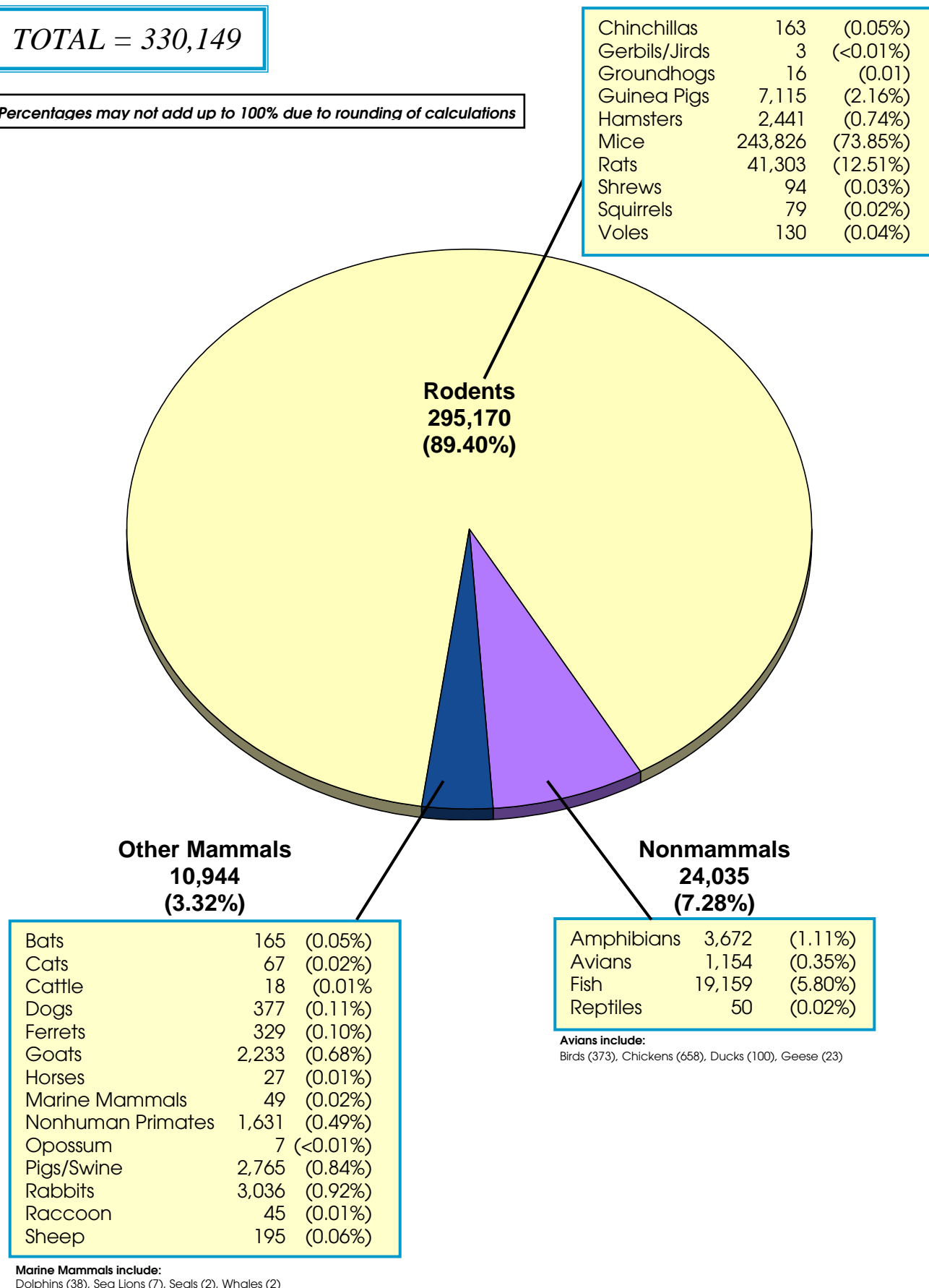
The majority of dogs (78%) and cats (74%) were used in medical research, including those in combat casualty care studies (34%), infectious disease research (16%), and medical training (8%). Dogs and humans are unique in their ability to develop prostate cancer and the former are studied in extramural prostate cancer research. Dogs are also a unique model for Leishmaniasis. Cats were used in neuroscience (75%) and training (25%).

A cornerstone of the IACUC animal use review process is to ensure the use of the lowest possible animal species on the phylogenetic scale. This has resulted in an overall drop in NHP, dog, cat, and general animal use since 1994. Compared to FY94, the use of dogs in FY01 is 59% lower (-551), cats 62% lower (-108), and NHPs 26% lower (-578). This illustrates the Department's continuing commitment to reducing the use of specific species in research.

DoD animal use by species is presented in Figure II-8. Figures II-9 and II-10 represent the intramural and extramural animal use by species, respectively, for FY01.

**TOTAL = 330,149**

Percentages may not add up to 100% due to rounding of calculations



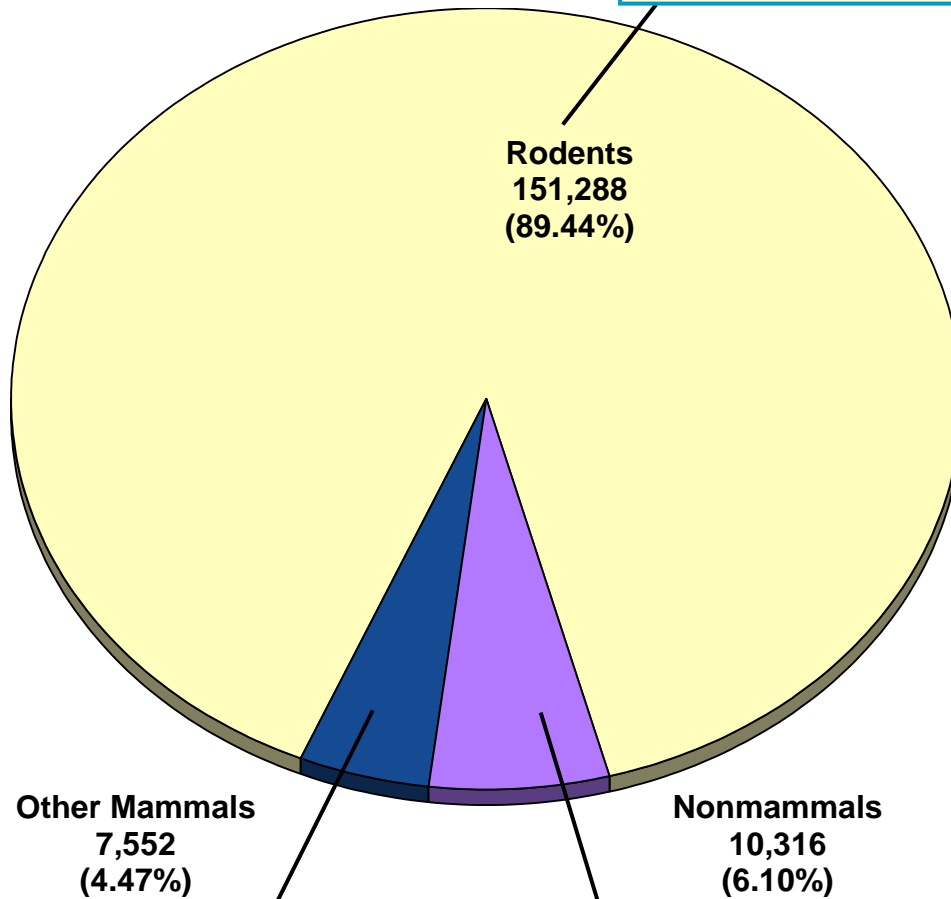
**Figure II-8** DoD Intramural and Extramural Animal Use by Species for FY01



***TOTAL = 169,156***

*Percentages may not add up to 100% due to rounding of calculations*

Chinchillas	163	(0.10%)
Gerbils	2	(<0.01%)
Groundhogs	16	(0.01%)
Guinea Pigs	6,485	(3.83%)
Hamsters	1,849	(1.09%)
Jird	1	(<0.01%)
Mice	123,952	(73.28%)
Rats	18,597	(10.99%)
Shrews	94	(0.06%)
Voles	129	(0.08%)



Bats	160	(0.10%)
Cats	17	(0.01%)
Dogs	93	(0.06%)
Ferrets	224	(0.13%)
Goats	2,233	(1.32%)
Horses	27	(0.02%)
Marine Mammals	33	(0.02%)
Nonhuman Primates	1,311	(0.78%)
Pigs/Swine	1,774	(1.05%)
Rabbits	1,592	(0.94%)
Sheep	88	(0.05%)

**Marine Mammals include:**  
Dolphins (30), Sea Lions (2), Whales (1)

Amphibians	460	(0.27%)
Avians	610	(0.36%)
Fish	9,242	(5.46%)
Reptiles	4	(<0.01%)

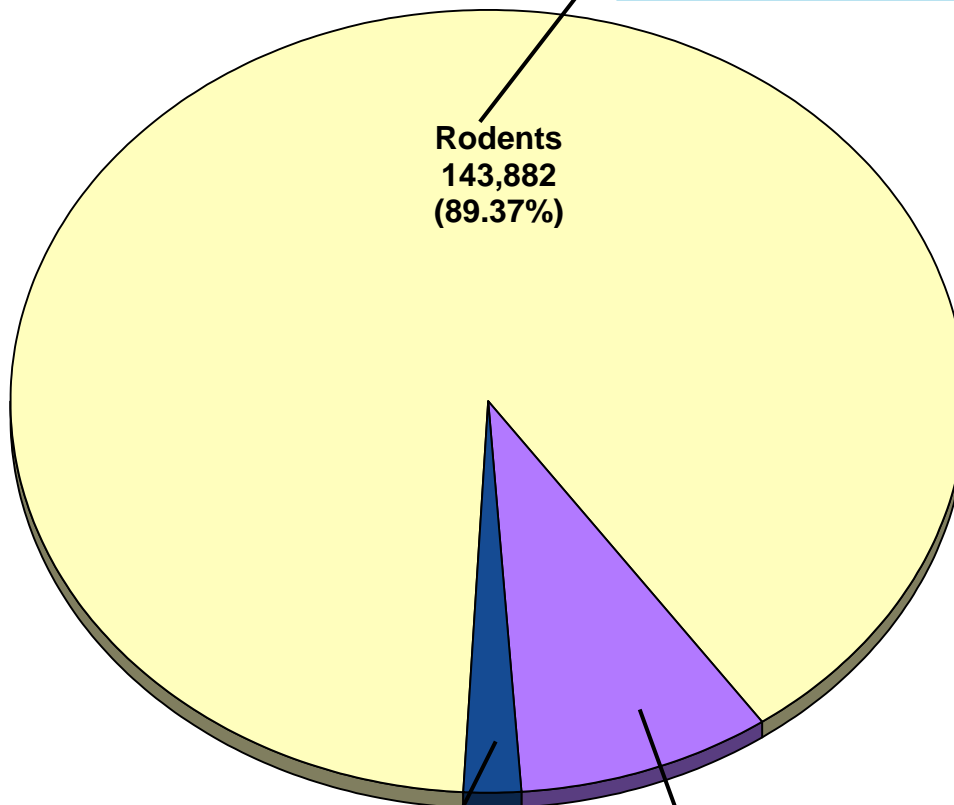
**Avians include:**  
Birds (373), Chickens (114), Ducks (100), Geese (23)

**Figure II-9** DoD Intramural Animal Use by Species for FY01

***TOTAL = 160,993***

*Percentages may not add up to 100% due to rounding of calculations*

Guinea Pigs	630	(0.39%)
Hamsters	592	(0.37%)
Mice	119,874	(74.45%)
Rats	22,706	(14.10%)
Squirrels	79	(0.05%)
Voles	1	(<0.01%)



Bats	5	(<0.01%)
Cats	50	(0.03%)
Cattle	18	(0.01%)
Dogs	284	(0.18%)
Ferrets	105	(0.07%)
Marine Mammals	16	(0.01%)
Nonhuman Primates	320	(0.20%)
Opossum	7	(<0.01%)
Pigs/Swine	991	(0.62%)
Rabbits	1,444	(0.90%)
Raccoons	45	(0.03%)
Sheep	107	(0.07%)

**Marine Mammals include:**  
Dolphins (8), Sea Lions (5), Seals (2), Whales (1)

Amphibians	3,212	(2.00%)
Avians	544	(0.34%)
Fish	9,917	(6.16%)
Reptiles	46	(0.03%)

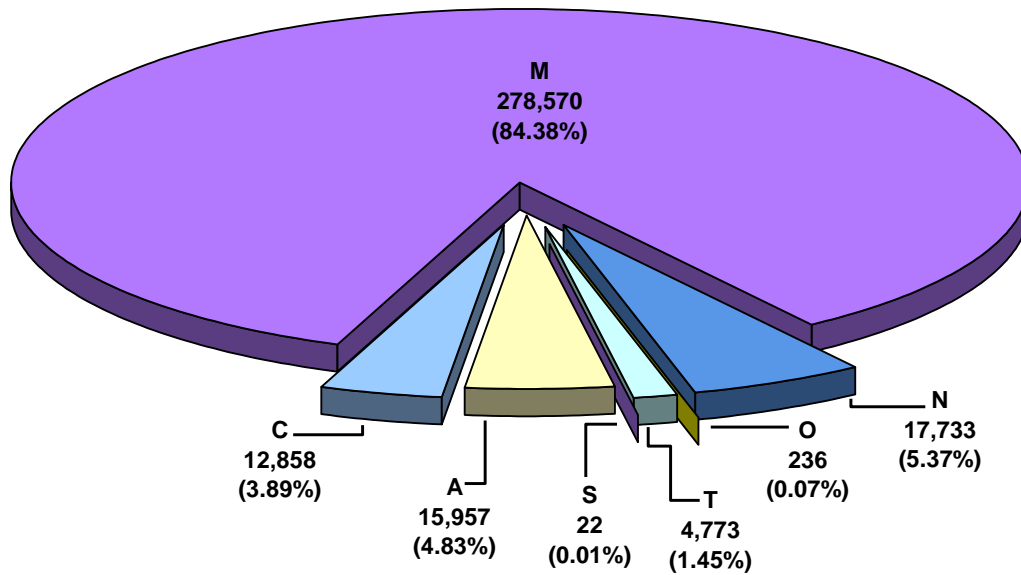
**Avians include:** Chickens (544)

**Figure II-10** DoD Extramural Animal Use by Species for FY01

## II.2.4 Animal Use by Animal Use Category

Total reported animal use in the DoD by animal use category is presented in Figure II-11, with the intramural and extramural breakouts in Figures II-12 and II-13, respectively.

***TOTAL = 330,149***

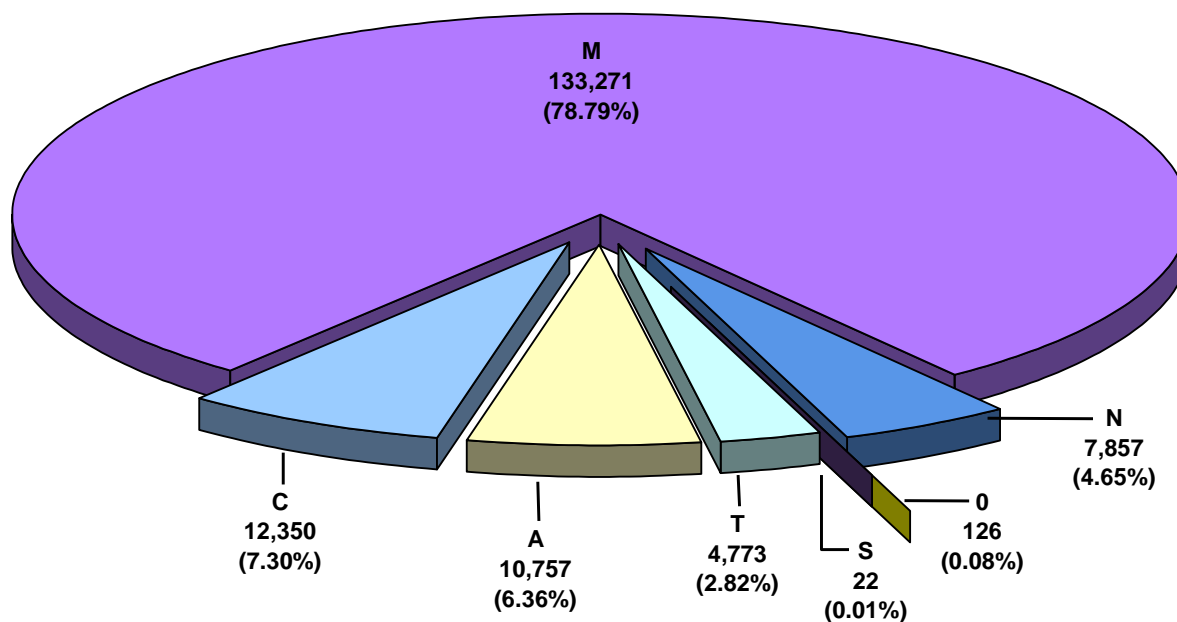


**Figure II-11** DoD Intramural and Extramural Animal Use by Animal Use Category for FY01

A: Adjuncts/Alternatives to Animal Studies, C: Clinical Investigations, M: Medical RDT&E, N: Nonmedical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations.

**TOTAL = 169,156**

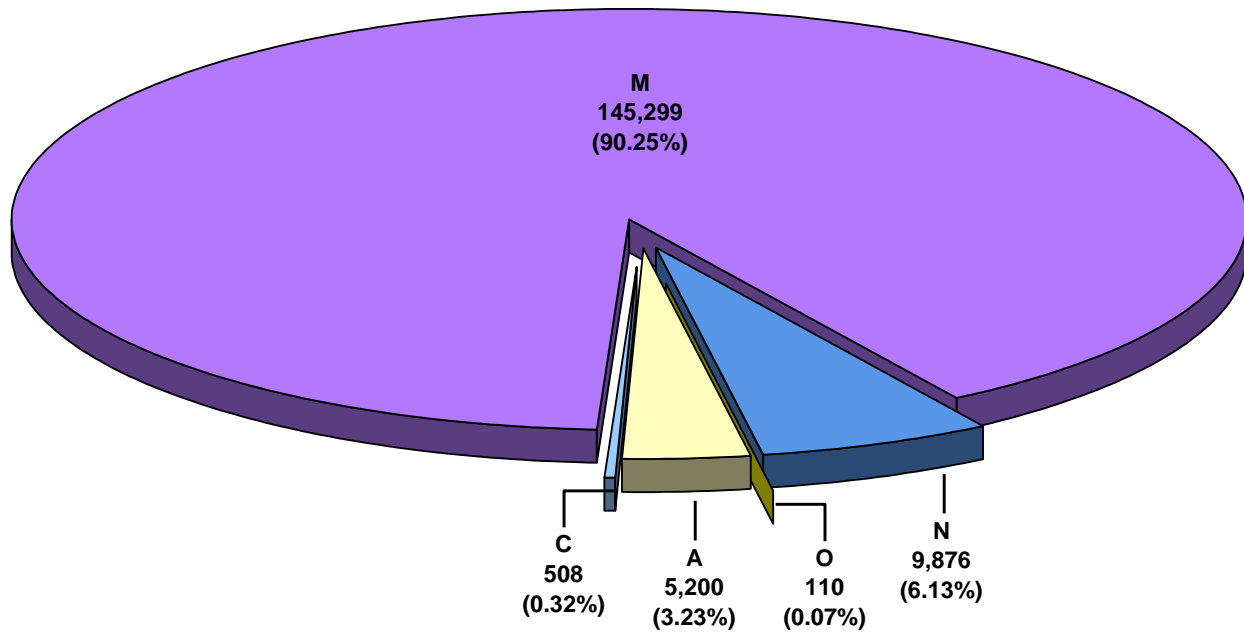


**Figure II-12** DoD Intramural Animal Use by Animal Use Category for FY01

A: Adjuncts/Alternatives to Animal Studies, C: Clinical Investigations, M: Medical RDT&E, N: Nonmedical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations.

***TOTAL = 160,993***



**Figure II-13** DoD Extramural Animal Use by Animal Use Category for FY01

A: Adjuncts/Alternatives to Animal Studies, C: Clinical Investigations, M: Medical RDT&E, N: Nonmedical RDT&E, O: Other Animal Use, S: Classified Secret or above, T: Training & Instructional.

Percentages may not add up to 100% due to rounding of calculations.

The DoD has a critical and challenging mission: to discover, design, and develop military countermeasures against threats to the health and survivability of military personnel. To meet this mission, 84% of the animals used by the DoD in FY01 were in medical RDT&E. Figure II-14 shows the breakout by medical subcategories. Twenty-five percent (83,132) of the animals used in medical RDT&E were in the area of infectious diseases (M2) and of those, 98% were rodents. The primary thrust of this research is the development of preventive measures against infectious disease through discovery, design, and development of prophylactic, therapeutic, and treatment drugs for relevant diseases. The chemical defense research program (M3) used 4% (11,693) and the biological defense research program (M4) used 14% (44,698) of the medical RDT&E animals. The Medical/chemical defense program is conducted to develop improved pretreatments, therapeutics, and diagnostics to protect the warfighter from exposure to chemical warfare agents. Medical/biological defense program is conducted to develop, demonstrate, and field new vaccines, drugs, and diagnostic kits for the prevention, treatment, and diagnosis of biological warfare agents such as anthrax. This research program protects the armed forces from the consequences of exposure to biological warfare agents and enhances their survivability. It has also assumed a central role in homeland defense and the development of countermeasures such as anthrax vaccines to terrorist threats. M4 animal use dropped by 38% relative to FY00, a decrease partially attributable to the development of refined protocols requiring fewer animals.

Research subcategories M5, M6 and M7 showed a combined decrease of 16% (-4142) from FY00. M5 research addresses the bioeffects of laser exposure, blast overpressure, operational stress and occupational health protection. M6 research is directed at combat casualty care issues such as the development of blood substitutes, and therapies for resuscitation, hemorrhage, shock and tissue injury. M7 studies are targeted at characterizing the effects of and treatment against, exposure to ionizing radiation.

M8 (Other Medical RDT&E) accounted for 35% of the total medical RDT&E category (Figure II-14). The CDMRP shown in Table II-3 used just over 111,000 animals in FY01. These programs, which are managed by the Army, are primarily directed at cancer biology and account for 96% of M8 animals (Table II-5), 38% of the animals used in medical RDT&E, and 34% of the total DoD animals used. These types of research programs can cause fluctuations in the total number of animals used from year to year depending on congressional funding levels and direction. Other areas of research within M8 are shown in Table II-5.

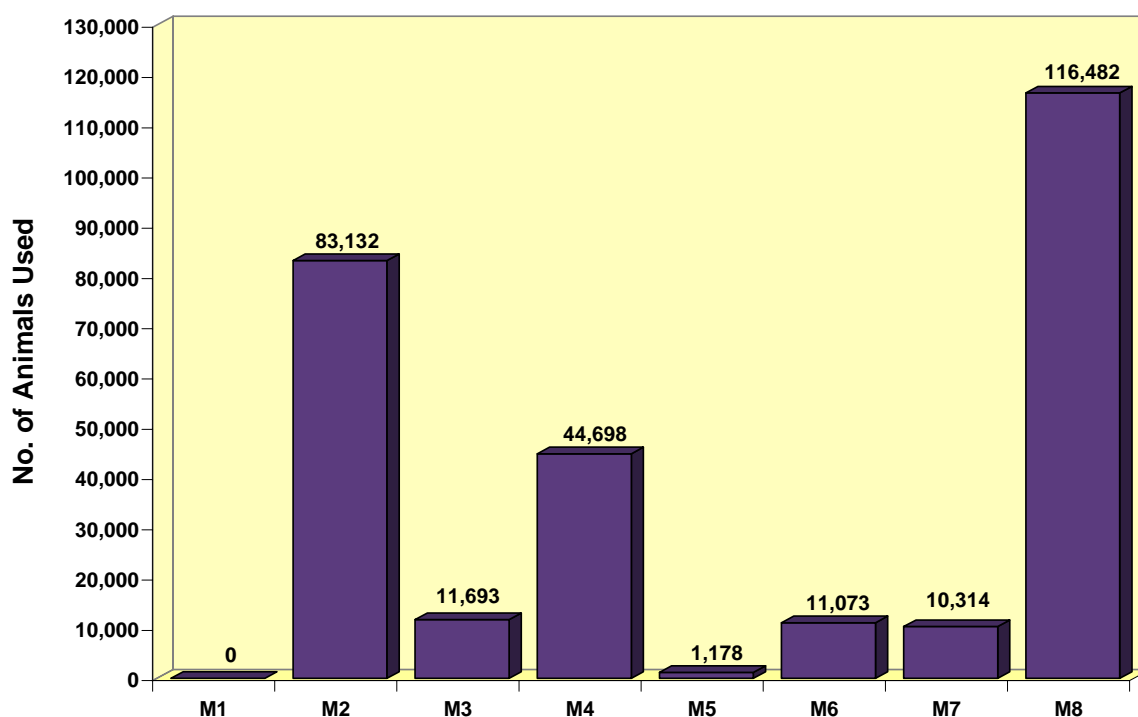


Figure II-14 Animal Use by Subcategories of the Medical Category

**Table II-5** Breakout of Animals Used in "Other Medical RDT&E" (Subcategory M8)

Results		
Research Category (M8)	Animals Used	% of M8
Bone Health Research	5,246	4.50%
Breast Cancer Research	57,382	49.25%
Defense Women's Health Research	152	0.13%
Environmental Safety	16	0.01%
Gulf War Illnesses	2,184	1.87%
Laser Research	34	0.03%
Neurofibromatosis Research	5,674	4.87%
Neurotoxin Research	12,946	11.11%
Occupational Medicine	6	0.01%
Ovarian Cancer Research	1,973	1.69%
Prostate Cancer Research	25,695	22.05%
Tissue-Based Sensors	98	0.08%
Toxicology	703	0.60%
Zoonosis	536	0.046%
Other Medical RDT&E	3,837	3.30%
<b>TOTAL</b>	<b>116,482</b>	<b>100%*</b>

\*Percentages may not add up to 100% due to rounding of calculations

Clinical investigations (Animal Use Category C) accounted for approximately 4% (12,678) of the animals used by the DoD in FY01. Studies in this category address clinical medicine and surgical problems for the treatment of both diseases and combat casualties. Sixty-two percent of the animals used in clinical investigations were used in clinical medicine studies. While many of these conditions are unique to the military, several are not.

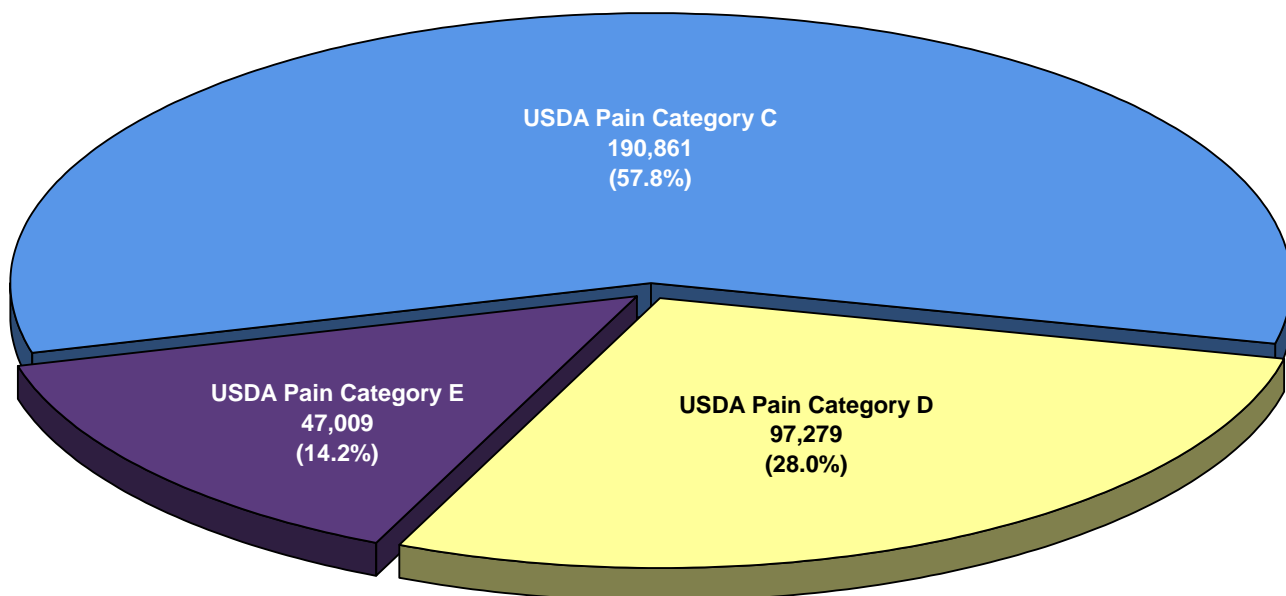
Nonmedical RDT&E animal use (Category N) accounted for slightly more than 5% (17,733) of the total FY01 animal use. Study in the area of Adjuncts/Alternatives to Animal Studies (Category A) accounted for 5% of the total animal use for FY01 and illustrates the Department's continuing effort to promote research to develop alternatives to reduce, replace, and refine the use of animals in DoD research. Less than 2% (4,773) of the animals used by the DoD in FY01 were in the training, education, and instruction of personnel (Training/Instructional Category T).

### II.2.5 Animal Use by USDA Pain Category

Total reported animal use in the DoD by USDA pain category is presented in Figure II-15, with the intramural and extramural breakouts in Figures II-16 and II-17, respectively.



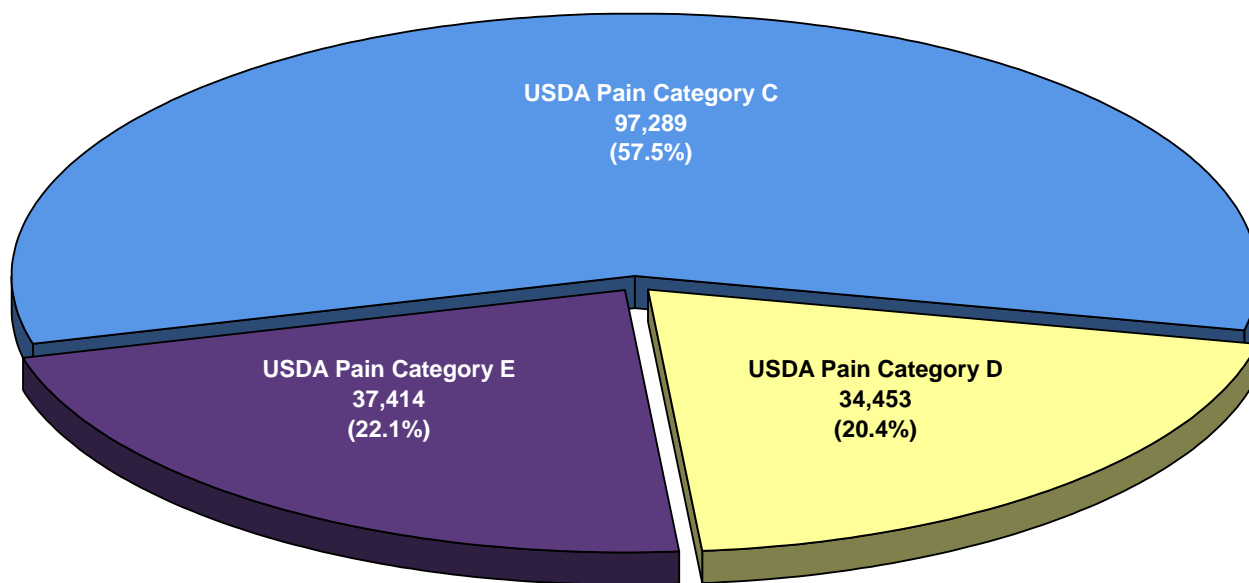
**TOTAL = 330,149**



**Figure II-15** DoD Intramural and Extramural Animal Use by USDA Pain Category for FY01

Percentages may not add up to 100% due to rounding of calculations

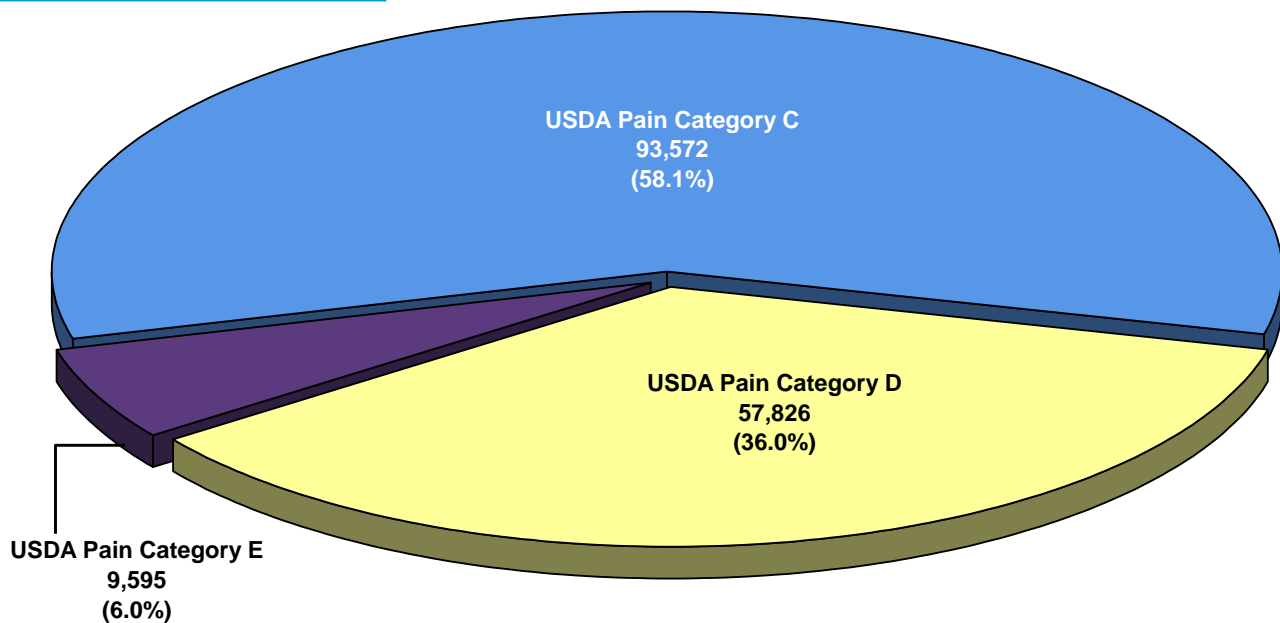
**TOTAL = 169,156**



**Figure II-16** DoD Intramural Animal Use by USDA Pain Category for FY01

Percentages may not add up to 100% due to rounding of calculations

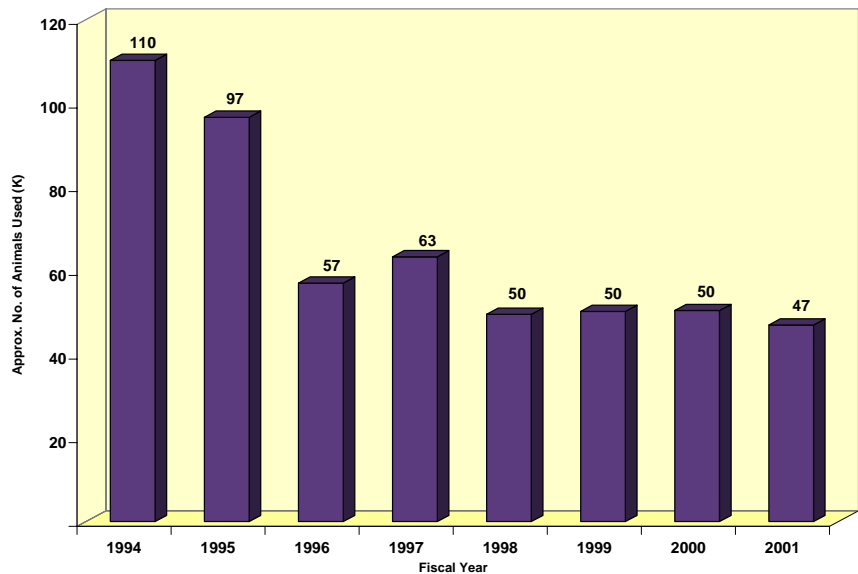
***TOTAL = 160,993***



**Figure II-17** DoD Extramural Animal Use by USDA Pain Category for FY01

*Percentages may not add up to 100% due to rounding of calculations*

The majority (94%) of DoD-supported research employing any species of animal was not painful to the animals involved. In most cases (58%), the animals were not exposed to or involved in any painful procedures (USDA Pain Category C). In 36% of the cases, animals were given anesthesia or pain-relieving drugs during procedures that might otherwise have involved pain or distress to the animals (USDA Pain Category D). In 6% of the animals used, anesthetics or analgesics were not used because they would have interfered with the validity of the results of experiments (USDA Pain Category E). Since FY94 the use of animals in Pain Category E has decreased steadily resulting in an overall decrease of 57% (63,273) (Figure II-18). A majority (89%) of the animals used in painful experiments (where reducing the pain or distress would have interfered with the validity of the results) were rodents. Fish accounted for 9% of the animals in USDA Pain Category E and other mammals accounted for 1% of animals in this pain category. Eighty-seven percent of the animals reported in USDA Pain Category E were used in medical studies; of these, 69% of the animals were used in research on infectious disease and chemical and biological defense and 19% in ionizing radiation studies. There were no animals subjected to unalleviated pain in the Training/Instructional category.



**Figure II-18** USDA Pain Category E by Fiscal Year

The DoD clearly has a most diverse, unique, and demanding RDT&E mission. The modern battlefield is a hostile and dangerous environment with extraordinary potential for exposure to lethal or debilitating conventional weapons, exotic endemic diseases, biological and chemical agents, nuclear blast and radiation, directed energy sources, and complex and dangerous equipment. In addition, a host of adverse environmental conditions, such as cold, heat, high and low pressure, and G-forces are threats to service men and women. The DoD must provide acceptable protection against these threats and many others. The animals reported in USDA Pain Category E were used in research designed to find ways to protect service men and women from the threats they encounter daily.

## SECTION III

# DoD INITIATIVES TO PROMOTE ALTERNATIVE METHODS THAT REPLACE, REDUCE, AND REFINE THE USE OF ANIMALS

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Alternatives, as articulated in *The Principles of Humane Experimental Technique* (Russell and Burch, 1959), are defined as methods that Replace, Reduce, and Refine the use of animals. In addition to these three Rs, the DoD advocates a fourth R, “Responsibility,” for implementing these alternative methods.

### **Replacement**

The replacement alternative addresses supplanting animal use with nonliving systems, analytical assays, cell-culture systems, and with animals that are lower on the phylogenetic scale. It includes the elimination of animal use altogether, generally by adopting in vitro or theoretical model study systems. Replacement also includes the substitution of species that are higher on the phylogenetic scale with those that are lower.

### **Reduction**

Reduction is the use of fewer animals without loss of scientific test validity. Decreasing the number of animal subjects through the use of statistical or innovative design strategies, while preserving the scientific integrity of the biological model, is a major emphasis of the reduction alternative to animal use.

### **Refinement**

Refinement is a procedure or measure taken to eliminate or minimize pain or distress in the animal(s) or enhance well-being while maintaining or improving the quality/quantity of research data collected. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, the use of adjusted early experimental endpoints, and the improvement of quality-of-life in animal housing.

### **Responsibility**

The DoD has taken responsibility for implementing animal use alternatives. It is reflected by the Department’s efforts to replace, reduce, and refine animal use in the context of ensuring scientific validity, study needs, and animal well-being. Department policy with regard to animal alternatives is promulgated in DoD Directive 3216.1, which directs that “it is DoD policy that alternatives to animal species should be used if they produce scientifically satisfactory results.” This policy is implemented in the Joint Regulation on the Use of Animals in DoD Programs, which delegates responsibility to the local commander for utilization of alternatives to animals.

To illustrate the Department’s initiatives to promote these four Rs, a description of such initiatives within DoD’s research laboratories and medical treatment centers is provided. The lists included in this section are not all inclusive, as the number of specific examples of implementing alternative methods that can be documented for DoD’s research projects is extensive. Rather, it illustrates the scope, diversity, and spirit of DoD’s four Rs initiatives. This section will demonstrate a broad-based movement toward the use of biotechnology and other innovative adjuncts to replace and reduce animal use as well as refinement in methods used in essential animal studies.

### III.1 DOD INITIATIVES TO PROMOTE ANIMAL ALTERNATIVES

The DoD has established a variety of initiatives and targeted programs that are currently in place to promote alternative methods that will replace, reduce, and refine the use of animals. These programs are designed to target individual and institutional awareness by providing educational opportunities, professional training, and fiscal resources toward implementing the four Rs approach to animal use.

#### III.1.1 Research to Reduce Reliance on Animals

The DoD continues to seek alternatives to animal use through a research objective initiated in FY93 entitled “Reducing Reliance on Human and Animal Subjects of Research and Improving Experimental Conditions Using Animals.” The purpose of this objective plan is to conduct basic research to develop new technologies to incrementally reduce future reliance on research animals. The U.S. Army Medical Research and Materiel Command’s Medical Biological Defense Research Program budgeted approximately \$175,000 in FY01 for this objective, which is available to support alternatives to animal use in research.

#### III.1.2 DoD-Sponsored Conferences and Workshops on Alternatives to Animal Use

Since 1990 the DoD has been promoting responsibility for alternatives to animal use by sponsoring major meetings and conferences on the subject. Every 2 years the DoD sponsors an international meeting on Alternatives to Animal Testing (Table III-1). These biennial conferences have been sponsored by the U.S. Army Soldier and Biological/Chemical Command (SBCCOM) and such prestigious cosponsors as the National Institute of Environmental Health Sciences (NIEHS), the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM), the U.S. Navy, the U.S. Air Force, Xenogen Corporation, the Gillette Company, the Humane Society of the United States, DermTech International, Interagency Committee on Neurotoxicology, Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the National Capital Area Chapter - Society of Toxicology, and the Association of Government Toxicologists.

**Table III-1** DoD-Sponsored Conferences

1990	DoD Initiatives in Alternatives to Animal Testing
1992	Current Concepts and Approaches on Animal Test Alternatives
1994	Alternatives in the Assessment of Toxicity: Theory and Practice
1996	Biennial International Symposium on Alternatives in the Assessment of Toxicity Issues, Progress and Opportunities
1998	Alternative Toxicological Methods for the 21st Century: Protecting the Human Health and Advancing Animal Welfare
2000	Alternative Toxicology Methods for the New Millennium—Science and Application

The 1992 meeting had 35 scientific platform sessions and 22 scientific poster presentations. This international symposium was attended by nearly 300 military and civilian scientists from four countries. Proceedings of the 1992 symposium are available through the Defense Technical Information Center (DTIC). In addition, in 1994 a book edited by Dr. Harry Salem entitled *Animal Test Alternatives* was published by Marcel Dekker, Inc., which included chapters prepared by most of the presenters at this symposium.

The 1994 meeting had 26 scientific platform sessions, including one by Dr. Martin Stephens of the Humane Society of the United States, and 45 scientific poster presentations. This meeting was attended by over 330 military and civilian scientists from seven countries. The proceedings and a monograph based on this successful symposium are available through DTIC. *Advances in Animal Alternatives for Safety and Efficacy Testing* was published by Taylor and Francis.

The 1996 conference was coordinated with the Scientists Center for Animal Welfare, which held its meeting June 10-11, 1996 to present Animal Welfare and Toxicology/Safety Studies: Current Issues and Trends for the Next Century. In December 1998, a DoD-supported meeting entitled, Alternative Toxicological Methods for the 21st Century: Protecting the Human Health and Advancing Animal Welfare conference was held in Bethesda, Maryland. This conference was sponsored by the SBCCOM, the CHPPM, the USAMRICD, and the NIEHS. Its purpose was to present the latest research and trends in programs to replace, reduce, or refine the use of research animals.

In November 2000 the 6th Biennial Alternatives Symposium entitled Alternative Toxicology Methods for the New Millennium - Science & Application was held at the Lister Hill Center, National Library of Medicine in Bethesda, Maryland. It provided 3½ days of cutting-edge scientific presentations and over 30 poster presentations. Papers were presented by researchers from SBCCOM, USAMRICD, CHPPM, Edgewood Chemical/Biological Center, DARPA, U.S. Navy, U.S. Air Force, Yale University, Harvard University, The Johns Hopkins University, Massachusetts Institute of Technology, the University of Michigan, the Food and Drug Administration, and the Environmental Protection Agency.

### *III.1.3 National Research Council, Institute of Laboratory Animal Research, Educational Programs*

The Department's priority and continuing commitment to promoting individual and institutional responsibility for alternatives to animal use are reflected in continuing financial support of the Institute of Laboratory Animal Research (ILAR) educational program of the National Research Council. The principal thrust of the ILAR grant is development of institutional training materials, educational courses, and publications in support of the Department's laboratory animal care and use programs. This ILAR information is used in various military research facilities as an important adjunct to existing investigator training and technical education programs on animal care and use. The ILAR information and programs have generated strong animal alternative provisions for both civilian and military-specific research opportunities. The Department has resolved to maintain this important collaboration in FY01 by providing in excess of \$141,000 for the ILAR program.

### *III.1.4 Institutional Animal Care and Use Committee Emphasis*

Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare), Parts 1-4 of the *Code of Federal Regulations* has specific provisions for addressing the issue of alternatives during the research animal protocol review process. The DoD has been a leader in constituting and operating IACUCs at its biomedical research facilities. Accordingly, DoD IACUCs consider alternatives to the proposed use of animals as an important review consideration. All DoD programs use a standardized [IACUC protocol format](#) for animal use proposals, which requires that nonanimal alternatives be considered. It states, "No study using animals should be considered prior to the elimination of all reasonable possibilities that the question might be adequately answered using other than animal means." Investigators must provide information on the animal model being proposed and justification for the selected species. The instructions for the Standard Protocol Format states, "investigators should use the least sentient species that will permit the attainment of research objectives." In addition, the investigators are required to provide a short description of the features of the proposal that may qualify the study as one that replaces, reduces, or refines the use of animals. The [DoD 1995 Policy Memorandum](#) requires that extramural contractor proposals utilizing animals in research, education, testing, or training include all the information contained in the DoD Standard Protocol Format, thereby requiring them to also provide the alternatives information.

## **III.2 DOD EXPERTISE AND TRAINING PROGRAMS THAT PROMOTE ANIMAL ALTERNATIVES**

### *III.2.1 Veterinary Staff Expertise and Assistance Visits*

The DoD component oversight offices each have credentialed LAM veterinarians who act as advisors to commanders on issues related to animal welfare and alternatives to animal use, and provide oversight to the

command's animal care and use programs. These officers make periodic assistance visits to the laboratories wherein they address the consideration of animal use alternatives.

### *III.2.2 Professional Veterinary Training in LAM*

The individuals who are specialty trained in veterinary LAM provide expertise in DoD biomedical research institutions, which strongly correlates to effective animal use alternative programs. An important responsibility of the LAM veterinarian is to review extramural animal use protocols, ensuring that alternatives to animal use and personnel training issues have been addressed. In FY01, 28 board-certified specialists of the American College of Laboratory Animal Medicine (ACLAM) served in the DoD. The DoD sponsors formal postdoctoral training programs for veterinarians in LAM, including a nationally recognized, 4-year program consisting of 2 years of residency training and 2 years of practical experience, culminating in specialty board eligibility for certification by the ACLAM. In August 1995, the DoD began a formal postgraduate Master's of Public Health in LAM at the USUHS.

The DoD has long been a leader in training veterinarians in the field of LAM, the biomedical and veterinary specialty most closely associated with laboratory animal welfare and laboratory animal care and use programs. Many of the nationally prominent leaders of several laboratory animal associations, such as the American Association for Laboratory Animal Science (AALAS), the American Society of Laboratory Animal Practitioners, and the ACLAM were formally trained in, or closely associated with, DoD LAM training programs. This strength in LAM expertise strongly enhances both animal care and use and animal alternative programs.

### *III.2.3 AALAS Technician and Laboratory Animal Science Training*

A number of DoD research facilities sponsor training programs leading to certification of animal care and research personnel as AALAS laboratory animal technicians. Individual DoD institutions have sponsored formal seminars for research personnel where experts from the National Agricultural Workshop present formal training and information on alternatives to animal use. In addition, the Walter Reed Army Institute of Research (WRAIR) offers quarterly workshops on ethical and administrative issues in animal use. Both the AALAS technicians' course and WRAIR workshop curriculum include formal training and information on animal use alternatives.

### *III.2.4 DoD Publications of Animal Use Alternatives*

DoD experts have published several documents on animal use alternatives in toxicology (referenced above). A book, entitled *Alternative Toxicological Methods for the New Millennium* is in press (CRC Press) and will be out by late 2002 or early 2003.

## **III.3 DOD IMPLEMENTATION OF ANIMAL USE ALTERNATIVES**

DoD research protocols strive to minimize animal use. During IACUC protocol review, investigators are specifically asked to present information indicating that "Reduction, Replacement, and Refinement" alternatives have been addressed.

In addition to the implementation of alternatives in research protocols, the DoD has established policies specific to the refinement of animal use in general animal housing and maintenance. For example, WRAIR has established a policy that mandates consideration of environmental enrichment for research animals. This policy allows for flexibility and creativity for improving conditions of laboratory animals. In FY01, animals assigned to 573 projects benefited from the implementation of environmental enrichment strategies. Environmental enrichment can include the provision of toys, increased housing space, or social housing strategies.

DoD animal use alternatives are categorized as "general" and "specific." General alternatives are frequently implemented in many different DoD programs. They include some standard practices, such as the statistical minimization of animal use for each protocol and other practices that are strongly encouraged through the IACUC review process. Specific alternatives are more unique than their general counterparts. They could be relevant



only to a single protocol or to a single facility. However, some of these represent new innovations that can be expected to disseminate to more widespread use in the research community. In FY01, the 35 intramural institutions reported over 775 general animal use alternatives. Eight institutions reported animal use replacement by eliminating the use of animals in 28 projects by implementing alternatives. Projects from 19 institutions were reported to reduce the number of animals required for training by using nonanimal training aids.

The following examples are a representative listing of general alternative methods reported by DoD facilities:

**Replacement**

- During the review process, all potential methods of adequately answering a research objective are reviewed prior to the use of an animal model.
- The evaluation process also considers the selection of a particular animal type; species lower on the phylogenetic scale are considered and used if their selection permits attainment of the research objectives.
- Nonanimal training aids are used to replace the use of live animals.
- Computer simulations are used in lieu of live animals when scientifically possible.

**Reduction**

- Animal use protocols are subject to review by a biostatistician who addresses the animal used, study design, and statistical evaluation packages, and ensures that the minimum number of animals will be used to meet the specific scientific objectives.
- Pilot studies are used to refine techniques and define the animal model so that animal use can be kept to the minimum required for statistical significance.
- Sharing of animal tissues among investigators reduces animal use.
- Iterations of the experiments are combined when possible to reduce the number of control animals used.
- Collaboration between DoD investigators or instructors allows for a single animal to be used in multiple training or research procedures and the sharing of control group information, resulting in an overall reduction in the number of animals used.
- Several types of data are collected simultaneously.
- Training sessions are designed to use the highest practical student-to-animal ratio.
- When possible, animals serve as their own controls.
- Studies are deliberately phased so they continue only if warranted.

**Refinement**

- Parameters developed for early or alternative endpoints are used as experimental endpoints when possible.
- Animals are anesthetized before euthanasia to decrease stress.
- Moribund animals are humanely euthanized to prevent unnecessary pain or distress.
- Utilizing the environmental enrichment strategy, animals are housed in social settings (i.e., pairs or groups) in an enriched environment (e.g., nest boxes and toys).
- Animal-handling skills and clinical techniques are taught to animal technicians, investigators, and research assistants to increase or ensure a proper skill level is attained prior to the start of a protocol.
- All Advanced Trauma Life Support training laboratory procedures are performed while the animals are under general anesthesia, and they are euthanized without regaining consciousness.

### III.4 DoD DEVELOPMENT OF ANIMAL USE ALTERNATIVES

FY01 DoD research shows that DoD organizations are actively involved in the development of alternatives to animal use. These developments have occurred through research specifically designed to produce alternatives and to improve experimental techniques. Whenever possible, DoD investigators attempt to develop state-of-the-art, scientifically relevant, and reliable experimental procedures that can be performed without the use of animals. In addition, in cases where the animal models cannot be completely replaced, investigators and veterinary staffs work diligently to develop refinement techniques to minimize animal pain and distress and improve the quality and quantity of data through the use of technology. The DoD is very active in the development of alternatives to the use of animals in research. Below are examples of specific alternatives developed and implemented by the DoD in FY01. This is only a sample of the alternatives development that was completed this year.

#### *III.4.1 Examples of Specific Alternatives Employed During FY01*

##### **Replacement**

Replacement Using in vitro Cell Cultures:

- Antiviral drugs are initially tested in vitro for their ability to inhibit flavivirus replication. Drugs that do not show activity in vitro are not tested in mice.
- Systems to harvest osteoclasts from the bone marrow of euthanized pigs, euthanized for other purposes rather than from live mouse pups, were developed.
- In vitro studies were performed to identify bacterial strains that produce desired antigens, and purified antigens were assessed using tissue cultures instead of mice.
- In rabies virus detection, mouse inoculation has been completely replaced with neuroblastoma cell culture isolation and Polymerase Chain Reaction amplification.
- In studies of cytotoxic T lymphocyte responses to alphavirus replicons expressing filovirus proteins, cloned cells are used instead of mice.
- In vitro cell culture assays specific to RNA challenge, viruses are used as part of the overall evaluation of the viruses in question.

Replacement Using Nonmammalian Species or Species Lower on the Phylogenetic Scale:

- Development of a mouse model of preconditioning has replaced the use of pigs in studies of ischemia.
- Pigs have replaced NHPs in studies of Staphylococcus infection.
- A model for dental implant research using pigs instead of dogs has been developed.
- Pharmacological studies employing visually stimulating testing and cognitively challenging behavioral tests are being conducted in mice and rats instead of macaques.
- Development of a mouse model of preconditioning has replaced the use of pigs in studies of ischemia.

Biochemical/Physical Methods and Other Technologies:

- Antibody yield expected from one cow is estimated to be equivalent to that produced by 10,000 mice; a nonpainful procedure in a single cow can replace a procedure that requires a large number of mice.
- Pig's feet purchased from a local grocery store are used to teach skin biopsy and suturing techniques, replacing live animal use.

##### **Reduction**

Substitution of Computer Simulation, Models, or Other Technologies:

- Once a hybridoma is produced (using a single mouse) that hybridoma can be grown in tissue culture as a source of antibody, thus obviating the need to immunize additional mice.

- Intubation is practiced using endotracheal models prior to utilizing anesthesia-teaching protocols
- The Resus-a-Pup mechanical model is used to teach canine CPR.
- The number of monkeys is reduced through the use of in vitro viral tissue culture infectivity titers instead of in vivo titers from monkeys.
- Predictive computational models reduce the use of rats by helping to screen for chemical toxicity.
- Installation of advanced caging systems and technology reduces the number of animals required to monitor colony infections.
- Use of an in vivo imaging device capable of identifying very small tumor nodules (less than 200 cells) in prostate cancer research reduced use of mice by 75%.
- Mannequins are used in training to give the students some experience in establishing airways before using pigs, reducing the number of animals required.
- Artificial rat models help surgical residents develop their microvascular and microneural surgical skills before they move on to live animals.
- In vitro cell culture-based protocols have been developed to allow for activity screening in vaccine development and reduce the number of mice required for testing.
- Physiologically-based pharmacokinetic computer modeling to investigate toxicity reduces the number of animals needed to complete the toxicological assessment.
- In dental readiness training, mannequins are used to give students experience in establishing airways, requiring fewer pigs.
- Using primary neuronal cell cultures markedly reduces the total number of rats required for neuroprotection experiments because multiple culture dishes can be prepared single rat embryos.
- A computerized system was developed for data acquisition and the control of the hemorrhage protocol. This allows for the collection of large amounts of data with reduced error and animal use.

#### Experimental Strategies:

- In studies of *Staphylococcus* enterotoxin B, the selection of inbred strains of mice has reduced variability and resulted in a requirement of fewer animals to achieve statistical validation.
- Prescreening in mice yields dose-ranging data that reduces the subsequent number of NHPs required for *Brucella* vaccine testing.

### Refinement

#### Reduce Pain and Distress:

- Propagation of the *Leishmania* organism in the tail of the gerbil greatly reduces the apparent distress experienced in previous studies employing the foot.
- A noninvasive, tail-cuff method to measure blood pressure has replaced an invasive method in rats.
- Aspirin was infused just prior to the infusion of saline or platelets to alleviate pain in rabbits.
- Surface and subdermal electrodes were used so that rats would not have to undergo surgery or live with electrodes implanted into their heads for long-term electrophysiological follow-up after a nontoxic exposure to chemical agent.
- Radiotelemetry was used to monitor physiological parameters in a number of projects involving rats and NHPs, reducing stress.
- Blood pressure oscillation has been found to be the earliest marker of light anesthesia in hemorrhaged rats and additional anesthesia is now routinely given to prevent sensation.
- Designed and constructed a hanging three-dimensional environmental enrichment center for the cat colony consisting of a series of baskets, ledges, hammocks, and toys. This added mental, physical, and visual variation to the cat room while at the same time providing required horizontal resting surfaces.
- Intravenous injections of small amounts of hemoglobin-containing liposomes represent a refinement over other models in which large amounts of blood substitute is infused in rats.
- Physiologic variables were measured under anesthesia, eliminating any stress during recording and manipulation and made available for various future analyses.

- Transcutaneous immunization of mice omits the need for needles and the pain associated with injection.
- Surgical procedures were changed to reduce the risk of nerve damage and muscle injury in rabbits.

### *III.4.2 Examples of Alternatives Undergoing Development During FY01*

As an ongoing process, the DoD is continuously developing alternatives. Below are examples of alternatives that were reported as currently in development by the DoD during FY01. This is only a sample of the alternatives being developed this year.

#### **Replacement**

Replacement Using in vitro Cell Cultures:

- To study Interactions of Liposomes with Mouse Macrophages investigators are trying to establish specific cell lines and clones which could replace live mice in the future.
- In vitro culture methods are being developed to allow cultivation directly from cryopreserved samples without the requirement for primate infection.
- Researchers are determining the suitability of replacing primary cells with macrophage-like, commercially available cell lines.

Non-Mammalian Species or Species Lower on the Phylogenetic Scale:

- The startle response of the bluegill sunfish is being investigated as an alternative to conventional neurotoxicologic tests that use rats.
- Pharmacological studies employing visually stimulating testing and cognitively challenging behavioral tests are being conducted in mice and rats instead of macaques.
- Development of the adult frog model to support the reproductive toxicity program.
- Membrane feeding techniques are being further optimized to reduce the number of animals necessary to maintain mosquito colonies.
- The use of brine shrimp to replace mice for the ricin lethality model is being explored.

Replacement with Computer Simulation, Models, or Other Technologies:

- Research continues to develop sonar and signal processing methods to replace marine mammals.
- A noninvasive snake model is being developed to replace the rhesus monkey in retinal research.

#### **Reduction**

Substitution of Computer Simulation, Models, or Other Technologies:

- In vaccine development, the identification of immunological target epitopes by computer searching eliminates the trial and error of finding appropriate targets, thereby reducing the number of experiments that fail.
- Ophthalmology training is being accomplished by replacing the use of rabbits with bovine eyes acquired from a local slaughter house. Researchers are developing an alternative, nonlethal model for demyelinating disease.
- Instructors are developing a course for pelvic surgery using cadavers instead of goats.
- The number of monkeys is being reduced through the use of in vitro viral tissue culture infectivity titers instead of in vivo titers from monkeys.
- The use of membrane blood feeding is being further developed as an alternative to mice in order to maintain mosquito and mite colonies as a source of biological material to conduct studies on the transmission of malaria, dengue, and scrub typhus.
- Investigation of gene expression profiles in cell cultures exposed to toxic chemicals is anticipated to enhance in vitro toxicity testing and reduce numbers of animals needed.

- The use of cuprizone toxin is being developed as a second model of demyelinating disease. This model does not cause fatality and does not have variable levels of severity based on sex and estrus cycle, leading to a reduced requirement for mice.

#### Utilization of Alternative Biological Testing Methods:

- Isolated rat hepatocytes are being used to screen the toxicity of chemicals using five different endpoints. Data are being analyzed to develop quantitative structure-activity relationships toward evaluating new chemicals and reducing the number of animals required to assess their toxicity.
- Molecular and computer methods from such areas as genomics, proteomics, and bioinformatics are being applied to evaluate the cellular responses from hundreds of known genes. By identifying molecular changes, these powerful tools could greatly reduce the use of animals in assessing therapeutic efficacy.
- Initial work is being performed in bacterial cultures toward determining the efficacy of laser wavelength and photosensitizers.
- Isolated, perfused rat livers are being characterized toward developing reliable models for drug distribution and metabolism. This is anticipated to diminish toxicity-related pain and distress and require fewer animals.
- Protocols for the isolation of insect-borne hepatitis and West Nile viruses using chicken serum and cell cultures are being developed toward reducing the need for mice.
- 

### **Refinement**

#### Environmental Enrichment:

- Development of environmental enrichment for NHPs by engaging them in behavioral interaction that emulates the essential features of natural foraging. The results will be used to further refine the environmental condition of captive NHPs and ensure their psychological well-being.
- Novel strategies and methods for improved environmental enrichment are being evaluated for many different animals.

#### Increased Training for Research Personnel to Improve Skills:

- Development of training programs to teach research personnel the technical skills necessary to properly manage and humanely handle NHPs during research experiments.
- Instruction in the care, handling, and management of rodents and lagomorphs.
- Development of veterinary techniques training programs for personnel utilizing various laboratory animal species will result in better animal handling.
- Training in surgical and aseptic techniques results in shorter surgery duration, less tissue trauma, and decreased post-operative complications.

#### Reduce Pain and Distress:

- Morphological changes in the snake retina following laser exposure are being studied toward developing a pain-free, noninvasive method employing the confocal scanning laser ophthalmoscope.
- In vaccine research, protocols are being developed that employ a surrogate marker to predict death, instead of employing death as an endpoint, will reduce unnecessary stress.
- Researchers are searching for an alternate adjuvant other than Freund complete for the use of high production of monoclonal antibodies will reduce animal discomfort and distress.

### **III.5 DoD PARTICIPATION IN OTHER FEDERAL ANIMAL ALTERNATIVE PROGRAMS**

The National Institutes of Health Revitalization Act of 1993 (Public Law (PL) No. 103-43, Section 1301) directed the NIEHS of the NIH to establish an Applied Toxicological Research and Testing Program, which represents the NIEHS' component of the National Toxicology Program. The Act further directed the NIEHS to "(a) establish criteria for the validation and regulatory acceptance of alternative testing methods, and (b) recommend a process through which scientifically validated alternative methods can be accepted for regulatory use." To fulfill this mandate, an ad hoc ICCVAM was established in 1994 by the NIEHS. In 2000, PL 106-545, the ICCVAM Authorization Act of 2000, established ICCVAM as a permanent committee. The mission of ICCVAM is to coordinate issues throughout the federal government that relate to the development, validation, acceptance, and harmonization of toxicological test methods. The ICCVAM is responsible for the coordination of the development and review of various alternative toxicological methods. The ICCVAM must also facilitate communication among all stakeholders in the development and review process of alternative methods. The ICCVAM evaluates proposals for alternative test methods and recommends further research. It comprises 47 members representing 15 different U.S. federal agencies. Members serve as points of contact and as sources to identify technical experts from their agencies to serve on specific topical workgroups. Recommendations regarding the usefulness of test methods provided by ICCVAM enable U.S. federal agencies to assess risks entailed by various test methods and make regulatory decisions. During FY01, the DoD had several representatives on the ICCVAM variously representing the U.S. Army Edgewood Research Center, the U.S. Army Center for Environmental Health Development Laboratory, the U.S. Air Force, DoD Tri-Service Toxicology Laboratory, and the Deputy Under Secretary for Science and Technology. The ICCVAM determines which assays warrant peer review, forms working groups, and supports test method workshops. When the members of the ICCVAM agree that an alternative method merits investigation, a working group is assembled. The working group in turn determines whether sufficient information exists for the assembly of either a peer review or a test method workshop. The results generated by ICCVAM's working groups may be used to recommend U.S. federal regulations and/or guidelines for research. More information on ICCVAM can be found at <http://iccvam.niehs.nih.gov/>.

### **III.6 SUMMARY**

Each year new techniques and capabilities improve the handling, treatment, and use of animals in RDT&E and training and potentially reduce the need for animals in those same endeavors. In FY01, there was significant evidence of the DoD's aggressive pursuit to develop alternatives to replace, reduce, and refine the use of animals (examples are highlighted in Section III.5). In addition to these developmental efforts, animal use data for FY01 indicate the widespread implementation of validated alternatives. Fish and frogs are replacing the use of many mice and rats while rats and mice continue to replace NHPs and other mammals higher on the phylogenetic scale in vaccine and drug development efforts. The number of large animals used by the military departments over the past decade has been significantly reduced, and some large species are rarely used at all. Dogs, cats, and NHPs have collectively decreased by 30% from FY94 to FY01 and represent less than 1% of the total animals used in research by the DoD. These and other examples of the development and implementation of alternatives have translated into reductions in the overall use of animals higher on the phylogenetic scale. Animal use alternatives including reduction, replacement, and refinement constitute key initiatives in the biomedical RDT&E, and educational training programs of the DoD.

This section can only partially document the persistent, ongoing efforts of DoD institutions to implement internal policies driving the refinement, reduction and replacement of animals used in training and laboratory research. Just as the DoD exceeds AWA reporting requirements in accounting for animal use, this Department exceeds external, federal regulations and policies governing the humane treatment of animals. The DoD mandates its animal use oversight bodies to review each protocol under consideration to ensure the implementation of the most favorable animal use alternatives in both animal maintenance and research. However, this spirit is carried even further forward with DoD-wide initiatives that are clearly demonstrated by commitments

to scientific research, initiatives and conferences specifically targeted at developing and implementing new animal use alternatives in refinement, reduction and replacement.

## **APPENDIX A**

### **DoD DIRECTIVE ON ANIMAL USE**





# Department of Defense DIRECTIVE

NUMBER 3216.1

April 17, 1995

Certified Current as of December 1, 2003

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DDR&E

SUBJECT: Use of Laboratory Animals in DoD Programs

- References: (a) DoD Directive 3216.1, "Use of Animals in DoD Programs," February 1, 1982 (hereby canceled)
- (b) Title 9, Code of Federal Regulations, "Animals and Animal Products," Chapter 1, Subchapter A, "Animal Welfare," Parts 1, 2, and 3
  - (c) Public Law 101-511, Department of Defense Appropriations Act for Fiscal Year 1991, Section 8019, and Section 2241 of title 10, United States Code
  - (d) Sections 2131 through 2156 of title 7, United States Code, "The Laboratory Animal Welfare Act of 1966," as amended
  - (e) through (f), see enclosure 1

## 1. REISSUANCE AND PURPOSE

1.1. Reissues reference (a) to update policy governing activities using animals within the Department of Defense.

1.2. Designates the Secretary of the Army as the DoD Executive Agent to develop and issue Service regulations to implement this Directive.

## 2. APPLICABILITY

This Directive applies to the Office of the Secretary of Defense, the Military Departments, the Uniformed Services University of the Health Sciences, and the Defense Agencies (hereafter referred to collectively as the "DoD Components") that perform or sponsor activities using animals.

### 3. DEFINITIONS

Terms used in this Directive are defined in enclosure 2.

### 4. DoD POLICY

4.1. Federal statutes, regulations, and publications that provide national standards and guidance for the acquisition, transportation, housing, control, maintenance, handling, protection, treatment, care, use, and disposal of animals shall be applicable to all activities using animals. A summary of the applicable documents cited as references is in enclosure 3.

4.2. Animals shall be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with reference (b) unless specifically exempted from the licensing requirements stated in reference (b).

4.3. DoD organizations or facilities maintaining animals for use in research, testing or training shall apply for accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

4.4. Alternative methods to animal species shall be considered, whenever possible, if such alternatives produce scientifically valid or equivalent results to attain the research testing and training objectives.

4.5. The purchase or use of dogs, cats, or nonhuman primates in research conducted for developing biological, chemical, or nuclear weapons is prohibited.

4.6. The purchase or use of dogs, cats, or nonhuman primates for inflicting wounds from any type of weapon(s) to conduct training in surgical or other medical treatment procedures is prohibited (reference (c)).

4.7. DoD organizations or facilities wishing to hold training programs using animals, such as advanced trauma life support (ATLS) training programs, shall have the training protocol reviewed and approved by a duly constituted Institutional Animal Care and Use Committee (IACUC) in accordance with references (d) and (e) and paragraph 4.8. of this Directive to ensure the humane use of animals. DoD organizations or facilities conducting ATLS training that require housing of animals for short periods of time shall ensure adequate care and shall have the animal housing facilities inspected and approved by a veterinarian prior to receipt of the animals.

4.8. All proposals or protocols for animal experiments or demonstrations in RDT&E, clinical investigation, instructional, or training programs conducted or sponsored by a DoD organization or facility shall be reviewed and approved by a duly constituted IACUC composed of a minimum of five members. There shall be at least one non-scientific member on each IACUC. In addition, there also shall be a member who represents the general community interest and is non-affiliated with the facility sponsoring IACUC. The non-affiliated and the non-scientific membership can be filled by the same person. To ensure community representation at each meeting and inspection, an alternate to the non-affiliated member shall be designated for IACUCs having a single non-affiliated membership. Since the DoD IACUCs perform a Government function in an approval process and do not serve merely as an advisory body, the non-affiliated and the non-scientific member(s) to DoD IACUCs shall either be a Federal employee, with demonstrated commitment to the community or a consultant consistent with the requirements established by reference (f).

4.9. A headquarters-level administrative review shall be conducted for proposals involving the use of nonhuman primates conducted or sponsored by subordinate activities of the DoD Component for conformance with all applicable Federal regulations and policies. A DoD Component may delegate this responsibility to another DoD Component for purposes of efficiency and consolidation of functional offices.

4.10. The DoD Components shall coordinate and cooperate in the transfer of Government-owned nonhuman primates between facilities to maximize conservation and proper utilization.

4.11. Proposals intending to use chimpanzees must be further reviewed and approved by the Interagency Animal Model Committee, which coordinates national priorities for research utilization of this species.

4.12. The DoD Components that sponsor animal-based research, testing, and training under a DoD grant or contract shall ensure that:

4.12.1. All extramural research proposals using live animals shall be administratively reviewed by a DoD veterinarian trained or experienced in laboratory animal science and medicine before grant or contract award.

4.12.2. The most recent USDA inspection reports are provided or obtained for the facility under consideration for a research contract or grant using animals, and that during the term of the award, the most recent USDA inspection reports be reviewed on an annual basis.

4.12.3. A DoD veterinarian trained or experienced in laboratory animal science and medicine shall conduct an initial site visit to evaluate animal care and use programs at contracted facilities conducting DoD-sponsored research using nonhuman primates, marine mammals, dogs, cats, or proposals deemed to warrant review. The initial site visit shall occur within 6 months of when the facility has taken delivery of the animals under DoD contract or grant award. Any facility receiving a DoD-funded grant or contract for animal-based research shall notify the DoD Component sponsor and shall have a site inspection within 30 days of notification of loss of AAALAC accreditation for cause, or notification that the facility is under USDA investigation. Site inspections for cause shall evaluate and ensure the adequacy of animal care and use in DoD-sponsored programs, and provide recommendations to the sponsoring DoD Component about continued funding support of the research.

4.13. In the case of differences between the standards of care and use of animals as cited in enclosure 3, the most stringent standard shall apply.

4.14. Activities covered by this Directive that are performed or sponsored in foreign countries shall be conducted in accordance with applicable U.S. statutory requirements, and regulations and standards of the host country. If differences exist between U.S. and host country regulations or standards, unless prohibited by the host country, the more stringent standard shall apply.

4.15. While not specifically addressed in this Directive, ceremonial, recreational, and working animals, such as military working dogs, shall be treated in a humane manner.

4.16. Personnel with complaints of violation of this Directive shall report such violations to either of the following members of the organization or facility: The IACUC chairperson, the attending veterinarian, the facility Commander, or the Inspector General. The IACUC shall review and, if warranted, investigate all reports of complaints of animal use or noncompliance with 7 U.S.C. 2131-2 of reference (d), applicable Directives, and Regulations.

## **5. RESPONSIBILITIES**

5.1. The Director, Defense Research and Engineering under the Under Secretary of Defense for Acquisition and Technology or designee shall:

5.1.1. Issue policy and procedural guidance concerning animal use consistent with all applicable Federal regulations and policies.

5.1.2. Designate a DoD representative to the Interagency Research Animal Committee who is a veterinarian of appropriate rank or grade and experience, and preferably also a diplomate of the American College of Laboratory Animal Medicine.

5.1.3. Establish the Joint Technical Working Group (JTWG) to act as the central advisory committee to the Armed Services Biomedical Research Evaluation and Management (ASBREM) Committee on all matters on the care and use of animals for research, testing, clinical investigation, or training within the Department of Defense. The co-chairpersons of the ASBREM Committee shall designate the chairperson of JTWG.

5.2. The Heads of the DoD Components shall:

5.2.1. Establish appropriate mechanisms to monitor compliance with this Directive and applicable Federal statutes and regulations.

5.2.2. Establish offices or facilities that shall serve as reviewing or approving authorities of animal use proposals from subordinate activities and extramural facilities proposing research under contract or grant.

5.2.3. Provide members to JTWG, as required.

5.2.4. Designate the appropriate office(s) within the DoD Component that shall perform the headquarters-level administrative review of proposals requiring the use of nonhuman primates and shall serve as the office where exemptions under paragraph 4.2., above, may be approved.

5.2.5. Support, and as necessary, ensure the development of animal care and use training programs for researchers and members of the IACUC, and certification programs for all personnel involved in the care, use, and treatment of animals.

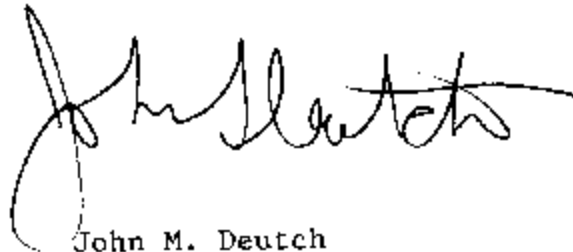
5.3. The Secretary of the Army shall:

5.3.1. As Executive Agent, develop and issue, in consultation with the other DoD Components, joint Service regulations to implement this Directive.

5.3.2. Designate the Commander, U.S. Army Veterinary Command/Director, DoD Veterinary Services Activity, a Field Operating Agency of the Army, Office of the Surgeon General, who shall serve as a consultant to the Assistant Secretary of Defense for Health Affairs and the Director, Defense Research and Engineering for technical and professional matters related to this Directive.

6. EFFECTIVE DATE

This Directive is effective immediately.

A handwritten signature in black ink, appearing to read "John M. Deutch". The signature is fluid and cursive, with a large initial "J" and "M".

John M. Deutch  
Deputy Secretary of Defense

Enclosures - 3

- E1. References, continued
- E2. Definitions
- E3. Guidance Documents

E1. ENCLOSURE 1

REFERENCES, continued

- (e) National Institutes of Health (NIH) Publication No. 86-23, "Guide for the Care and Use of Laboratory Animals," United States Department of Health and Human Services, National Institutes of Health, Revised 1985
- (f) Section 3109 of title 5, United States Code

## E2. ENCLOSURE 2

### DEFINITIONS

E2.1.1. Animal. Any dog, cat, non-human primate, guinea pig, hamster, rabbit or any other live vertebrate animal, which is being used or is intended for use for research, training, testing, or experimentation purposes. For this Directive, it includes birds, rats of the genus *Rattus* and mice of the genus *Mus* bred for use in research, training, testing or experimentation purposes. The term excludes animals used for ceremonial or recreational purposes, military working animals, and animals intended for use as livestock and poultry as food or fiber; or, livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber.

E2.1.2. Clinical Investigation. All activities directed towards clinical research conducted principally within medical treatment facilities. The Clinical Investigations program is part of the Defense Health Program of the Assistant Secretary of Defense (Health Affairs) and is supported by Major Force Program 8 (MFP-8) funds.

E2.1.3. Instructional Program. All educational and training activities, except training of ceremonial and recreational animals and training associated with military working animals or survival skills training.

E2.1.4. Research, Development, Test, and Evaluation. All activities that form the RDT&E program of the Director, Defense Research and Engineering (DDR&E) and are supported by Major Force Program 6 (MFP-6) funds.

E2.1.5. Alternatives. Any system or method that covers one or more of the following: replacing or reducing the number of laboratory animals required for an investigation by computer simulation, cell culture techniques, etc.; or, refining an existing procedure or technique to minimize the level of stress endured by the animal.

E2.1.6. DoD Sponsored Programs. All proposals or designs for animal experiments or demonstration in RDT&E, clinical investigation, or instructional programs conducted or funded by grant, award, loan, contract, or cooperative research and development agreement (CRADA).



### E3. ENCLOSURE 3

#### ADDITIONAL FEDERAL STATUTES, REGULATIONS, AND GUIDELINES ON THE USE OF ANIMALS

E3.1.1. The following documents provide national standards and guidance for the protection, treatment and use of animals:

E3.1.1.1. Animal Welfare Act (Sections 2131-2158 of title 7, United States Code, as amended, and Title 9, Code of Federal Regulations, Parts 1-4, implementing rules and regulations). Administered by Regulatory Enforcement and Animal Care (REAC), Animal and Plant Health Inspection Service (APHIS) of the Department of Agriculture. Requires licensing of dealers, identification of animals, maintenance of records, submission of reports, establishment of an Institutional Animal Care and Use Committee (IACUC), and compliance with standards for the humane handling, care, treatment, and transportation of animals by dealers and research facilities.

E3.1.1.2. Endangered Species Act of 1973 (Sections 1531-1543 of title 16, United States Code, as amended, and Title 50, Code of Federal Regulations, Parts 10-14 and 217-227, implementing rules and regulations). Provides a program under the U.S. Fish and Wildlife Service, Department of Interior, for conserving threatened and endangered species. Requires import/export permits, maintenance of records, and submission of reports on the care and handling of endangered, threatened, and conserved species.

E3.1.1.3. Marine Mammal Protection Act (Sections 1361-1384 of title 16, United States Code, as amended, and Title 50, Code of Federal Regulations, Parts 10-14 and 216-227, implementing rules and regulations). Provides a program under the Departments of Commerce (National Marine Fisheries Service) and Interior (U.S. Fish and Wildlife Service) for the protection of marine mammals and marine mammal products. Requires acquisition permits, maintenance of records, submission of reports, and inspections on the care and handling of marine mammals.

E3.1.1.4. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (TIAS 8249, as amended, and Title 50, Code of Federal Regulations, Part 23, implementing rules and regulations). CITES is a treaty involving 106 signatory nations administered in the United States by the Fish and Wildlife Service of the Department of the Interior. CITES regulates the import and export of imperiled species covered by the treaty but imposes no restrictions or control on interstate shipments.

E3.1.1.5. Lacey Act (Section 42 of title 18, United States Code, as amended, and Title 50, Code of Federal Regulations, Part 16 and Subpart B, implementing rules and regulations). A program under the U.S. Fish and Wildlife Service, Department of the Interior. Prohibits the importation of certain wild animals or their eggs if the Secretary of the Interior determines that they are injurious to humans, the interest of agriculture, or other specified national interests.

E3.1.1.6. Guide for the Care and Use of Laboratory Animals. Public Health Service, National Institutes of Health, NIH Publication No. 86-23, Revised. Provides guidelines for institutional policies, husbandry, requirements, veterinary care, and physical plant requirements for programs involving the care and use of laboratory animals.

E3.1.1.7. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Published by the Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 309 West Clark Street, Champaign, IL 61820, March 1988. Provides guidelines for the care and use of the major agricultural animal species in the United States in research and teaching.

## **APPENDIX B**

# **DoD POLICY FOR COMPLIANCE WITH FEDERAL REGULATIONS AND DoD DIRECTIVES FOR THE CARE AND USE OF LABORATORY ANIMALS IN DoD-SPONSORED PROGRAMS**



OFFICE OF THE SECRETARY OF DEFENSE

WASHINGTON, D.C. 20301

10 APR 1995

MEMORANDUM FOR ASSISTANT SECRETARY OF THE ARMY (M&RA)  
ASSISTANT SECRETARY OF THE ARMY (RDA)  
ASSISTANT SECRETARY OF THE NAVY (M&RA)  
ASSISTANT SECRETARY OF THE NAVY (RDA)  
ASSISTANT SECRETARY OF THE AIR FORCE  
(MRAI&E)  
ASSISTANT SECRETARY OF THE AIR FORCE (SAF/AQ)  
PRESIDENT, UNIFORMED SERVICES UNIVERSITY OF THE  
HEALTH SCIENCES  
DIRECTOR, DEFENSE NUCLEAR AGENCY  
DIRECTOR, ADVANCED RESEARCH PROJECTS AGENCY

SUBJECT: Department of Defense (DoD) Policy for Compliance with  
Federal Regulations and DoD Directives for the Care and  
Use of Laboratory Animals in DoD-Sponsored Programs

References:

(a) Title 7, United States Code, Sections 2131-2156,  
The Laboratory Animal Welfare Act of 1966, PL 89-544,  
as amended PL 94-279, 1976, and PL 99-198, 1985.

(b) Review of the Use of Animals in the Department of  
Defense Medical Research Facilities, Inspector General  
Department of Defense, February 1994.

(c) Review of the Use of Animals in Department of  
Defense Contract Research Facilities, Inspector  
General Department of Defense, August 1994.

Definition:

(a) Animal means any dog, cat, non-human primate, or  
any other live vertebrate animal which is being used  
or is intended for use for research, training, testing,  
or experimentation purposes. For this Policy Guidance,  
it includes birds, rats of the genus Rattus and mice of  
the genus Mus bred for use in research, training,  
testing or experimentation purposes. The term excludes  
animals used for ceremonial or recreational purposes,  
military working animals, and animals intended for use  
as livestock and poultry as food or fiber; or,  
livestock or poultry used or intended for use for  
improving animal nutrition, breeding, management, or  
production efficiency, or for improving the quality of  
food or fiber.

(b) DoD-Sponsored programs means any study, proposal,  
or design for animal experimentation or demonstration  
in Research Development, Test, and Evaluation (RDT&E),  
clinical investigation, or instructional program  
conducted or funded by grant, award, loan, contract, or  
cooperative research and development agreement (CRADA).

Reference (a) has been accepted by the Department of Defense (DoD) in the development of DoD Directives and policy guidance. References (b) and (c) contain recommendations which have been endorsed by the Department. The purpose of this policy memorandum is to implement the recommendations contained in references (b) and (c).

DoD components that utilize animals in DoD-supported programs shall be aware of the attached DoD Directive 3216.1, "Use of Laboratory Animals in DoD Programs," appended as attachment (1). It is currently pending signature and will supersede the current DoD Directive 3216.1 dated February 1, 1982. Additional policy guidance is as follows:

a) In DoD component facilities conducting animal-based programs, an alternate to the non-affiliated member of the Institutional Animal Care and Use Committee (IACUC) shall be designated for IACUCs having a single non-affiliated member. The non-affiliated member(s) or alternates must receive a minimum of eight hours training. At least four hours of the training shall address the regulatory responsibilities and proper techniques on animal protocol review processes. An additional minimum of four hours of training will address humane care and ethics issues dealing with animal use. All DoD Components conducting animal use programs as defined shall have training programs for non-affiliated IACUC members in place by 1 October 1995.

b) All DoD component facilities maintaining animals used in research, testing, or training shall apply for accreditation by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The Office of the Director, Environmental and Life Sciences, Pentagon Room 3D129, Washington, D.C. 20301-3030 is the central point of contact to maintain cognizance over the application or continuation of AAALAC accreditation. All DoD facilities shall furnish copies of AAALAC accreditation status to that office. Absence of accreditation shall be explained with a plan of action and milestones to obtain accreditation.

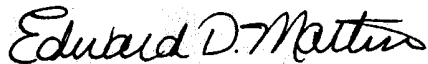
The following recommendations from the DoD Inspector General have been adopted as policy and shall be fully implemented by DoD Components which use animals in DoD-sponsored programs.

a) The DoD standard protocol format appended as attachment (2) shall be implemented by 1 October 1995. All intramural protocols involving animal use submitted after 1 October 1995 shall use the standard format. Extramural contractor proposal submissions need not use the standard format; however, the contractor shall provide all pertinent information contained in the standardized protocol format.

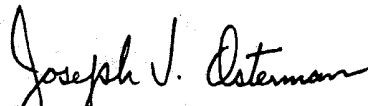
b) All DoD component facilities that utilize animals in research, testing and training shall implement the DoD standardized semi-annual program review checklist appended as attachment (3) immediately. Accompanying the checklist is a detailed outline of program review as contained in the NIH Guide for the Care and Use of Laboratory Animals. The Guide is the primary reference which is used by AAALAC in the accreditation process. The checklist shall be completed as a part of the semiannual IACUC program and facility review process. The semi-annual IACUC reports shall contain a copy of the checklist or indicate that the checklist was used as the basis of the program and facility review. A majority of members of the IACUC shall sign the report and include a statement indicating the presence or absence of minority opinions.

c) Commanders, and Directors of DoD component facilities shall support and, as necessary, develop animal care and use training programs for personnel associated with animal use programs, and encourage certification for all personnel involved in the care, use and treatment of laboratory animals.

As of 1 October 1995, DoD components shall report all animal-based protocols in the required format redacted for public release to the Defense Technical Information Center (DTIC). Selected fields of the DTIC report will be made accessible to the public through the INTERNET.



Edward D. Martin  
Principal Deputy,  
Assistant Secretary of  
Defense (Health Affairs)



Joseph V. Osterman  
Director, Environmental  
and Life Sciences

Attachments:

- (1) Pending DoD Directive 3216.1
- (2) Standard Protocol Format
- (3) Standard Semi-annual Checklist

## **APPENDIX C**

### **DoD STANDARD IACUC PROTOCOL FORMAT INSTRUCTIONS**

## Appendix C

### DOD Animal Use Protocol Format

#### C-1. Requirements

All DOD animal use protocols must use the format shown in this appendix. This protocol format includes requirements of the Animal Welfare Act Regulations, the Guide, and other applicable Federal regulations and DOD directives.

#### C-2. Protocol cover sheet

Before the protocol is submitted for IACUC review, at least three signatures are required on the protocol cover sheet (fig C-1). They must include those of the Principal Investigator (P.I.); either the department or division chief or the scientific review committee chairperson; and the individual performing the statistical review.

---

I. Name of Facility

II. Proposal Number

III. Title

IV. Principal Investigator(s)/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

V. Scientific/Division Review/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

VI. Statistical Review/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

VII. Attending Veterinarian/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

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Figure C-1. DOD animal use protocol cover sheet

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*a. Scientific/division review.* This signature verifies that the animal use proposal received appropriate scientific peer review and is consistent with good scientific practice.

*b. Attending veterinarian.* The Animal Welfare Act Regulations require that an attending veterinarian must be consulted in the planning of procedures/manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

*c. Statistical review.* A person knowledgeable in biostatistics is required to review all proposals to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.



### **C-3. DOD animal use protocol format**

*a.* The format shown in figure C-2 is designed to be used with several word-processing programs on a personal computer as a “fill-in-the-blank” type of document. It is available electronically through the appropriate DOD component oversight office listed in appendix B. Each paragraph and subparagraph in the format must have a response. Title headings do not require a response. Portions of the protocol format that are not applicable will be marked “N/A.” There are no space limitations for the responses. Pertinent standing operating procedures or similar documents that are readily available to the IACUC may be referenced to assist in the description of specific procedures.

---

PROTOCOL TITLE  
PRINCIPAL INVESTIGATOR(S)  
CO-INVESTIGATOR(S)  
I. NON-TECHNICAL SYNOPSIS  
II. BACKGROUND  
II.1. Background  
II.2. Literature Search for Duplication  
II.2.1. Literature Source(s) Searched  
II.2.2. Date of Search  
II.2.3. Period of Search  
II.2.4. Key Words of Search  
II.2.5. Results of Search  
III. OBJECTIVE/HYPOTHESIS  
IV. MILITARY RELEVANCE  
V. MATERIALS AND METHODS  
V.1. Experimental Design and General Procedures  
V.1.1. Experiment 1  
V.1.2. Experiment 2  
V.2. Data Analysis  
V.3. Laboratory Animals Required and Justification  
V.3.1. Non-animal Alternatives Considered  
V.3.2. Animal Model and Species Justification  
V.3.3. Laboratory Animals  
V.3.3.1. Genus and Species  
V.3.3.2. Strain/Stock  
V.3.3.3. Source/Vendor  
V.3.3.4. Age  
V.3.3.5. Weight  
V.3.3.6. Sex  
V.3.3.7. Special Considerations  
V.3.4. Number of Animals Required (By Species)  
V.3.5. Refinement, Reduction, Replacement  
V.3.5.1. Refinement  
V.3.5.2. Reduction  
V.3.5.3. Replacement  
V.4. Technical Methods  
V.4.1. Pain/Distress Assessment  
V.4.1.1. APHIS Form 7023 Information (See attending veterinarian for assistance)  
V.4.1.1.1. Number of animals  
V.4.1.1.1.1. Column C: \_\_\_\_ (Animal #)  
V.4.1.1.1.2. Column D: \_\_\_\_ (Animal #)  
V.4.1.1.1.3. Column E: \_\_\_\_ (Animal #)  
V.4.1.2. Pain Relief/Prevention  
V.4.1.2.1. Anesthesia/Analgesia/Tranquilization  
V.4.1.2.2. Pre- and Post-procedural Provisions  
V.4.1.2.3. Paralytics  
V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures  
V.4.1.3.1. Sources Searched  
V.4.1.3.2. Date of Search  
V.4.1.3.3. Period of Search  
V.4.1.3.4. Key Words of Search  
V.4.1.3.5. Results of Search  
V.4.1.4. Unalleviated Painful/Distressful Procedure Justification  
V.4.2. Prolonged Restraint  
V.4.3. Surgery

**Figure C-2. DOD animal use protocol format**

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- V.4.3.1. Pre-surgical Provisions
  - V.4.3.2. Procedure
  - V.4.3.3. Post-surgical Provisions
  - V.4.3.4. Location
  - V.4.3.5. Surgeon
  - V.4.3.6. Multiple Major Survival Operative Procedures
  - V.4.3.6.1. Procedures
  - V.4.3.6.2. Scientific Justification
  - V.4.4. Animal Manipulations
  - V.4.4.1. Injections
  - V.4.4.2. Biosamples
  - V.4.4.3. Adjuvants
  - V.4.4.4. Monoclonal Antibody (MAbs) Production
  - V.4.4.5. Animal Identification
  - V.4.4.6. Behavioral Studies
  - V.4.4.7. Other Procedures
  - V.4.4.8. Tissue Sharing
  - V.4.5. Study Endpoint
  - V.4.6. Euthanasia
  - V.5. Veterinary Care
  - V.5.1. Husbandry Considerations
  - V.5.1.1. Study Room
  - V.5.1.2. Special Husbandry Provisions
  - V.5.1.3. Exceptions
  - V.5.2. Veterinary Medical Care
  - V.5.2.1. Routine Veterinary Medical Care
  - V.5.2.2. Emergency Veterinary Medical Care
  - V.5.3. Environmental Enrichment
  - V.5.3.1. Enrichment Strategy
  - V.5.3.2. Enrichment Restriction
  - VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING
  - VII. BIOHAZARD/SAFETY:
  - VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the P.I. unless directed by the IACUC.
  - IX. ASSURANCES: The law specifically requires several written assurances from the Principal Investigator. Please read and sign the assurances as indicated.

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

**Figure C-2. DOD animal use protocol format—Continued**

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E. Training: I verify that the personnel performing the animal procedures/manipulations/observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R," namely "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL or WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(PRINT) First Name, MI, Last Name of Principal Investigator

Signature

Date (YYYYMMDD)

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**Figure C-2. DOD animal use protocol format—Continued**

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*b.* Some information may be added to the format to meet local needs. However, all labeled paragraphs and subparagraphs will remain in the same relative order. The added information will be similar or complementary to the information requested. Other types of requirements specific to a given Service, command, or locale (such as budgeting information, local coordinating requirements, or specific scientific review requirements, and so forth) can be added by placing them in front or behind the standard format.

#### **C-4. Protocol format with completion aids**

The format shown in figure C-3 is the same protocol format as in figure C-2. Explanations have been added to aid in completing the protocol proposal.

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PROTOCOL TITLE: Title must include species of animal(s) used in research.

PRINCIPAL INVESTIGATOR(S)  
CO-INVESTIGATOR(S)

I. NON-TECHNICAL SYNOPSIS: Provide a brief, narrative description of the proposal that is easily understood by a high school graduate. Include animal use in your description. (NOTE: This information may be used to complete the DOD Annual Report to Congress.)

## II. BACKGROUND

II.1. Background: Include a brief statement of the requirement or need for the information being sought. Lengthy explanations are not required. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited, and a description of the general approach will be provided.

II.2. Literature Search for Duplication: This search must be performed to prevent unnecessary duplication of previous experiments. A search of the Biomedical Research Database (BRD) is required. In addition, a search of EITHER the Federal Research in Progress (FEDRIP) OR the Computer Retrieval of Information of Scientific Projects (CRISP) database is required. Requirements for additional searches are at the discretion of the IACUC.

II.2.1. Literature Source(s) Searched

II.2.2. Date of Search

II.2.3. Period of Search

II.2.4. Key Words of Search

II.2.5. Results of Search: Provide a narrative description of the results of the literature search.

III. OBJECTIVE/HYPOTHESIS: State the objective of this protocol or the hypothesis to be accepted or rejected. (NOTE: This information will be used to complete the DOD Annual Report to Congress.)

IV. MILITARY RELEVANCE: Provide a brief and succinct military justification for the research with regard to military needs and mission requirements. If applicable, state the Science and Technology Objective (STO) that this work supports.

## V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: This section includes an explanation of experimental design. Technical methodology need not be described in this section, rather, it should be described under paragraph V.4, Technical Methods. Provide a complete description of the proposed use of animals to include a summary table of the experimental groups. Succinctly outline the formal scientific plan and direction of experimentation. If several experiments or sequential studies are to be included in the protocol, describe the experimental design of each separate experiment in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. A clearly understandable description of the numbers of animals and their distribution into experimental groups is essential. The number requested must equal the minimum number required to complete the study yet be sufficient to yield meaningful results. The minimum number includes animals necessary for controls or technique development, and so forth. Inclusion of a summary table or flow chart showing the distribution of animals by experimental group is highly recommended. The total number of animals required for the study is listed in section V.3.4.

V.1.1. Experiment 1

**Figure C-3. DOD animal use protocol format with completion aids**

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## V.1.2. Experiment 2

V.2. Data Analysis: List the statistical test(s) planned or describe the strategy intended to evaluate the data. Describe the statistical methodology used to determine group size and total number of animals. A power-based assessment of the sample size is the preferable method of determining the minimum number that is likely to yield significant results with given alpha and beta errors, estimated effect size and expected variability. Be certain to include animals necessary for controls or technique development, and so forth.

## V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: State all non-animal alternatives (for example, computer modeling, in vitro cell culture work) that were considered. Explain why animals are needed.

V.3.2. Animal Model and Species Justification: Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model? If less sentient (invertebrate versus vertebrate) animal models were considered but not chosen, explain why.

## V.3.3. Laboratory Animals

### V.3.3.1. Genus and Species

V.3.3.2. Strain/Stock: If inbred or specialized animals are required, use proper terminology. (See the attending veterinarian for assistance.)

V.3.3.3. Source/Vendor: Provide a preferred source for the animals. Animals will be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with Code of Federal Regulations, Title 9, Animals and Animal Products, Chapter 1, Subchapter A, Animal Welfare, Parts 1, 2, and 3. (See the attending veterinarian for assistance.)

### V.3.3.4. Age

### V.3.3.5. Weight

### V.3.3.6. Sex

V.3.3.7. Special Considerations: List specialized requirements for animals here (for example, simian immunodeficiency virus or herpes antibody free, Pasteurella free, and so forth).

V.3.4. Number of Animals Required (By Species): The number of animals stated here must correspond exactly to that described in section V.1. If, during the completion of the protocol, additional animals are needed owing to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval of additional animals.

V.3.5. Refinement, Reduction, Replacement (3 Rs): Investigators are required to consider the 3 Rs when preparing an animal use research protocol. In the paragraphs below, describe all provisions in this protocol that refine, reduce, or replace the use of animals. Discuss what provisions were considered and why they were not chosen. If N/A is used, explain why.

V.3.5.1. Refinement: Procedures or measures taken to eliminate or minimize pain or distress in the animal(s) or enhance animal well-being. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, or the use of adjusted early experimental endpoints. In addition to listing refinements, list

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Figure C-3. DOD animal use protocol format with completion aids—Continued

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refinement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.2. Reduction: Procedures or measures taken to reduce the number of animals used. Examples of reduction include but are not limited to the use of shared or historical control groups, preliminary screening in non-animal systems, and innovative statistical packages. In addition to listing reductions that will be used, list reduction alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.3. Replacement: Procedures or measures that eliminate the use of animals. Examples of replacements include but are not limited to the use of non-animal models or less sentient animal species. In addition to listing replacements that will be used, also list replacement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.4. Technical Methods: This information must be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure, obtain a clear understanding of what is to be done and how the animals will be handled, and make a reasonable determination as to whether this proposed use of laboratory animals is in compliance with DOD regulations, guidelines, and Federal law.

V.4.1. Pain/Distress Assessment: The law defines a painful procedure as one that would “reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied; that is, pain in excess of that caused by injections or other minor procedures.” If a procedure may involve pain or distress, even if relieved by anesthetics or analgesics, the P.I. must consult with the attending veterinarian.

V.4.1.1. APHIS Form 7023 Information: (See your attending veterinarian for assistance.) The protocol must contain an estimate of the number of animals that will be counted in columns C, D, and E of the APHIS Form 7023, Annual Report of Research Facility. Columns C, D and E represent specific pain categories. (See below paragraphs, V.4.1.1.1.1.-3.) The animal should be listed in the column corresponding to the most painful or distressful procedure experienced by the animal. It is possible for one protocol to have animals listed in several columns. For instance, control animals may be placed in Column C while experimental animals may be placed in Column D, depending upon the nature of the protocol. Reflect use of more than one species of animals in a duplicate table. The total numbers reflected in these three columns will add up to the number of animals requested for the entire protocol in paragraph V.3.4.

#### V.4.1.1.1. Number of Animals

##### V.4.1.1.1.1. Column C: \_\_ (animal #)

Examples of research procedures/manipulations that would require an animal to be placed in Column C are studies involving not more than slight or momentary pain and/or distress in a human being to which that procedure is applied.

##### V.4.1.1.1.2. Column D: \_\_ (animal #)

Examples of procedures/manipulations that would require an animal to be placed in Column D are procedures where anesthesia or analgesia will be administered to avoid or effectively relieve pain or distress. General anesthesia given for surgical procedures, or the use of analgesia or anti-inflammatory agents are examples of this category.

##### V.4.1.1.1.3. Column E: \_\_ (animal #)

Examples of procedures/manipulations that would require an animal to be placed in Column E are procedures in which alleviation of pain or distress are contraindicated for a scientifically justifiable reason such as the experimental results are likely to be confounded if drugs relieving pain or distress were

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Figure C-3. DOD animal use protocol format with completion aids—Continued

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administered. Detailed justification for putting animals into this category is required below in paragraph V.4.1.4.

#### V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Describe the methods or strategies planned to effectively relieve or prevent pain or distress if the study will cause more than slight or momentary pain or distress. If pain/distress relief/prevention is planned, specify agents to be used and when these agents will be given (pre-emptive or post-procedural). Provide agent, dosage, and frequency of administration.

V.4.1.2.2. Pre- and Post-procedural Provisions: Describe the provisions for both pre- and post-procedural care, including provisions for post-procedural observations and frequency of observations. (Information concerning pre- and post-surgical care should be listed in paragraphs V.4.3.1 and V.4.3.3). If analgesics are used for pain/distress relief, provide the frequency of administration, observational criteria utilized to determine if animals are experiencing pain or distress, and the location for the post-procedural care.

V.4.1.2.3. Paralytics: The use of paralytic agents without anesthesia is prohibited. Describe the monitoring method that will be used to ensure adequate depth of anesthesia while the animal is under the influence of the paralytic agent.

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures: Respond N/A if the animals will experience not more than momentary or slight pain or distress and are placed in column C of APHIS Form 7023. (See paragraph V.4.1.1.)

V.4.1.3.1. Source(s) Searched: Examples are AGRICOLA, MEDLINE, BIOSIS, Altweb, and so forth.

V.4.1.3.2. Date of Search

V.4.1.3.3. Period of Search

V.4.1.3.4. Key Words of Search: Examples are pain, surgery, alternatives, LD 50, analgesia, anesthesia, death as an endpoint, distress, species of animal(s) to be used, name of painful or distressful experimental procedure, and so forth.

V.4.1.3.5. Results of Search: Provide a narrative summary of the results of the literature search for alternatives. The Animal Welfare Act specifically states that the P.I. must provide a narrative description of the methods and sources, e.g., the Altweb (Johns Hopkins Center for Alternatives to Animal Testing), MEDLINE, Life Sciences Abstracts, AGRICOLA, and BIOSIS) that he/she used to determine that alternatives to the painful procedure were not available. Discuss alternatives (those that would meet your scientific objectives) considered but not chosen. The alternatives literature search MUST be performed even when animals are placed in Column D and the pain or distress is alleviated through the use of analgesics or anesthetics.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Procedures that cause more than slight or momentary pain or distress that is not alleviated through the effective use of anesthetics or analgesics must be justified on a scientific basis in writing by the P.I. This paragraph must be completed if there are ANY animals in this protocol that will experience unalleviated pain or distress.

V.4.2. Prolonged Restraint: Describe (period of restraint, method, and timing of animal observations, habituation/training of animal to restraint device) and justify in detail any prolonged restraint greater than 12 hours for nonhuman primates or in accordance with IACUC policy for other species. Examples of restraint methods are primate chairs, restraint boards, metabolism cages, and so forth. This section is not intended for short-term actions such as rabbit restraint for bleeding, and so forth.

V.4.3. Surgery: Major survival operative procedures on non-rodent species will be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions.

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Figure C-3. DOD animal use protocol format with completion aids—Continued

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Non-survival operative procedures do not require a dedicated facility, but they should be performed using surgical gloves, mask, and clean instruments. Additionally, the surgical site should be clipped and cleaned prior to surgery. Major survival rodent surgery does not require a dedicated facility but it must be performed using aseptic technique; that is, aseptic patient preparation, surgical gloves, mask, and sterile instruments. A major operative procedure is defined as a procedure that penetrates and exposes a body cavity, or causes substantial or permanent impairment of physical or physiological function.

V.4.3.1. Pre-Surgical Provisions: Describe the provisions for pre-surgical care, including provisions for pre-surgical observations and frequency of pre-surgical observations. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the pre-surgical care.

V.4.3.2. Procedure: Describe in detail any surgical procedures planned.

V.4.3.3. Post-Surgical Provisions: Describe the provisions for post-surgical care, including provisions for post-surgical observations, frequency of post-surgical observations and criteria for early euthanasia owing to surgical complications or pain that cannot be relieved. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the post-surgical care.

V.4.3.4. Location: Give the location/room number for the proposed surgical procedure.

V.4.3.5. Surgeon

V.4.3.6. Multiple Major Survival Operative Procedures: The principal investigator must scientifically justify multiple major survival operative procedures performed on the same animal.

V.4.3.6.1. Procedures

V.4.3.6.2. Scientific Justification

V.4.4. Animal Manipulations: Describe any injections, sampling procedures, or other manipulations of the animals necessary for the study. A reference or SOP may be furnished to the IACUC to document a particular procedure in lieu of a detailed description.

V.4.4.1. Injections: Information must include route of injection, dosage, frequency, volume injected, needle size, and anatomic injection site.

V.4.4.2. Biosamples: Examples include cerebrospinal fluid taps, blood sampling, and biopsies. List volumes taken, sampling site, frequency of sampling, needle size, and method of sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

V.4.4.3. Adjuvants: List any adjuvants used and the plan for their use. Provide a scientific justification for the use of Complete Freund's Adjuvant (CFA) and discuss why other less reactive adjuvants cannot be used. Provide dosages, volumes, route, number of injection sites, and injection locations. Specify frequency and method of injection site monitoring and include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.4. Monoclonal Antibody (MAbs) Production: Provide a scientific justification for *in vivo* MAbs production. What *in vitro* methods of MAbs production were considered but not used? For *in vivo* MAbs production, specify the priming agent, animal monitoring frequency, number and frequency of abdominal taps, and fluid replacement therapy. Include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.4.5. Animal Identification: Describe the method of animal identification used in this study. Examples include microchips, tattoos, ear tags, and cage cards.

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Figure C-3. DOD animal use protocol format with completion aids—Continued

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V.4.4.6. Behavioral Studies: Fully describe the use of aversive stimuli, food or water restriction, and so forth, that would affect the study animals. Include methods of monitoring physiologic or behavioral indexes, including criteria (for example, weight loss or state of hydration) for temporary or permanent removal of the animal from the study. Provide an appropriate scientific justification for this type of behavior modification. An IACUC policy may be included where applicable.

V.4.4.7. Other Procedures: Describe all procedures which have not been explained in other sections of this proposal that will be performed while conducting this research. Examples include electrocardiograms, radiology, and aerosol exposure.

V.4.4.8. Tissue Sharing: List what tissues will be shared, with whom, and for what purpose.

V.4.5. Study Endpoint: State the projected study endpoint for the animals (for example, recovery and return to issue pool, euthanasia, or death without early euthanasia). Indicate whether recovery, euthanasia, or death is expected; and the specific plan for determining when the animal experimentation phase will be stopped. The P.I. must ensure that unnecessary pain or distress is prevented by carefully considering "When is the experimental question answered?" so that the animals can be expeditiously removed from the study. Define specific criteria that will be used to determine study endpoint (for example, weight loss, loss of locomotion and significant lowering of body temperature, decreased food or water consumption, and decreased activity). Specifically address and scientifically justify any proposal in which critically ill or moribund animals are allowed to die as a result of the experimental procedures without the benefits of veterinary medical treatment or early euthanasia. Explain the plan for the disposition of surviving animals or animals removed from the study prior to its completion.

V.4.6. Euthanasia: If applicable, discuss the euthanasia method. The Animal Welfare Act defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current American Veterinary Medical Association (AVMA) guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. If requested, the attending veterinarian will assist in selecting the best method for euthanasia.

V.5. Veterinary Care: If requested, the attending veterinarian of the facility will assist PIs with preparing this section.

V.5.1. Husbandry Considerations: Federal regulations require that animal housing and living conditions must be appropriate to their species and contribute to their health and comfort. Briefly describe animal husbandry to include routine animal observations, caging methods, feed and water provisions, environmental parameters, sanitation schedules, and light cycles.

V.5.1.1. Study Room: Where will the experimental procedure be conducted? Will the animal be housed in this room for more than 12 hours?

V.5.1.2. Special Husbandry Provisions: Examples include micro-isolators, metabolic cages, food and water restriction.

V.5.1.3. Exceptions: Describe any deviations/exceptions to *The Guide for the Care and Use of Laboratory Animals*, the Animal Welfare Act regulations, or IACUC policy that have an impact on animal housing space, feeding, and sanitation. Deviations/exceptions must be justified by the P.I. and approved by the IACUC.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: Describe the routine veterinary medical care. State if the animals will be observed daily or more frequently. Indicate what will happen if the animal becomes ill or

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**Figure C-3. DOD animal use protocol format with completion aids—Continued**

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debilitated during the study and requires evaluation. List the criteria used for health evaluation while the animals are on study (for example, weight loss, ruffled fur, dehydration, decreased activity, and hunched body position). Include a response plan (for example, alternative early endpoint and veterinary medical treatment) in the event of debilitating illness or an adverse reaction.

V.5.2.2. Emergency Veterinary Medical Care: Describe emergency veterinary medical care.

### V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy: Discuss enrichment provided to animal species listed in this protocol.

V.5.3.2. Enrichment Restriction: Provide written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates. Single housing of nonhuman primates and dogs without sensory contact with conspecifics must also be justified and approved by the IACUC.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING: List the names, qualifications and training by procedure of all personnel working with animals assigned to this protocol. Personnel performing observations, procedures, and/or manipulations described in the protocol must be identified and appropriately trained and qualified to perform these procedures. Contact the attending veterinarian for assistance with this requirement.

VII. BIOHAZARD/SAFETY: Provide a list of any potential biohazards associated with the chosen animal model and this research proposal (for example, viral agents, toxins, radioisotopes, oncogenic viruses, and chemical carcinogens). Describe safety precautions and programs designed to protect personnel from biohazards associated with this research and any surveillance procedures in place to monitor potential exposures.

VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the PI unless directed by the IACUC.

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**Figure C–3. DOD animal use protocol format with completion aids—Continued**

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### **C–5. Personnel qualifications.**

*a.* A Study Personnel Qualifications/Training table must be included in section VI of the protocol description. The table format is preferred by the IACUC for ease of reviewing the protocol. The table will contain the following four column headings:

- (1) Name of the activity (for example, the procedure, observation, or manipulation to be performed, such as the venous catheterization of a dog).
- (2) Name of the person performing the activity.
- (3) Qualifications of the person performing the activity (for example, assistant laboratory animal technician (ALAT), 2 years experience).
- (4) Training of the person performing the activity (for example, Canine Procedures Workshop, 1999).

*b.* Itemize each activity being performed in the protocol. List per species if there are multiple species in the protocol. If more than one individual is performing the activity, list each individual separately.

## **APPENDIX D**

### **DoD SEMIANNUAL PROGRAM REVIEW AND FACILITY INSPECTION CHECKLIST**

## **Appendix D**

### **Instructions for Use of DD Form 2856 (DOD Semiannual Program Review/Facility Inspection Checklist)**

#### **D-1. The checklist and the inspection report**

The IACUC must complete the DOD Semiannual Program Review/Facility Inspection Checklist during the IACUC semi-annual program review and facility inspection in accordance with Title 9, Code of Federal Regulations, Subchapter A, Part 2, Subpart C. Individual checklists must be kept on file in the IACUC office but do not require attachment to the finished IACUC Semiannual Program Review/Facility Inspection Report.

#### **D-2. Use of the form**

The use of the form is self-explanatory; simply place a checkmark in the most appropriate category for each item on the inspection list. A sample completed DD Form 2856 is shown in figure D-1.

DOD SEMIANNUAL PROGRAM REVIEW/ FACILITY INSPECTION CHECKLIST				
ORGANIZATION	DATE OF REVIEW (YYYYMMDD)			
DOD Animal Facility	2002 Jul 19			
Completion of this checklist by the IACUC during the semi-annual program review and facility inspection is mandatory. Mark X in the most appropriate category for each item. KEY: A = Acceptable; M = Minor deficiency; S = Significant deficiency (is or may be a threat to animal health or safety).				
CATEGORIES	A	M	S	N/A
<b>SECTION I - INSTITUTIONAL POLICIES AND RESPONSIBILITIES</b>				
<b>1. MONITORING THE CARE AND USE OF ANIMALS</b>				
a. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE	X			
b. ANIMAL CARE AND USE PROTOCOLS	X			
c. PHYSICAL RESTRAINT	X			
d. MULTIPLE MAJOR SURGICAL PROCEDURES	X			
e. FOOD OR FLUID RESTRICTION	X			
<b>2. PERSONNEL QUALIFICATIONS AND TRAINING</b>	X			
<b>3. OCCUPATIONAL HEALTH AND SAFETY OF PERSONNEL</b>				
a. HAZARD IDENTIFICATION AND RISK ASSESSMENT		X		
b. PERSONNEL TRAINING	X			
c. PERSONAL HYGIENE	X			
d. FACILITIES, PROCEDURES, AND MONITORING	X			
e. ANIMAL EXPERIMENTATION INVOLVING HAZARDS	X			
f. PERSONAL PROTECTION	X			
g. MEDICAL EVALUATION AND PREVENTIVE MEDICINE FOR PERSONNEL	X			
<b>SECTION II - ANIMAL ENVIRONMENT, HOUSING, AND MANAGEMENT</b>				
<b>4. PHYSICAL ENVIRONMENT</b>				
a. MICROENVIRONMENT AND MACROENVIRONMENT		X		
b. HOUSING	X			
c. SPACE RECOMMENDATIONS	X			
d. TEMPERATURE AND HUMIDITY	X			
e. VENTILATION	X			
f. ILLUMINATION		X		
g. NOISE	X			
<b>5. BEHAVIORAL MANAGEMENT</b>				
a. STRUCTURAL ENVIRONMENT	X			
b. SOCIAL ENVIRONMENT	X			
c. ACTIVITY	X			
<b>6. HUSBANDRY</b>				
a. FOOD	X			
b. WATER	X			
c. BEDDING	X			
d. SANITATION	X			
e. WASTE DISPOSAL	X			
f. PEST CONTROL	X			
g. EMERGENCY, WEEKEND, AND HOLIDAY CARE	X			
<b>7. POPULATION MANAGEMENT</b>				
a. IDENTIFICATION AND RECORDS	X			
b. GENETICS AND NOMENCLATURE	X			

DD FORM 2856, AUG 2002

Figure D-1. Sample completed DD Form 2856

CATEGORIES	A	M	S	N/A
<b>SECTION III - VETERINARY MEDICAL CARE</b>				
<b>8. ANIMAL PROCUREMENT AND TRANSPORTATION</b>	X			
<b>9. PREVENTIVE MEDICINE</b>				
a. QUARANTINE, STABILIZATION, AND SEPARATION	X			
b. SURVEILLANCE, DIAGNOSIS, TREATMENT, AND CONTROL OF DISEASE	X			
<b>10. SURGERY</b>	X			
<b>11. PAIN, ANALGESIA, AND ANESTHESIA</b>	X			
<b>12. EUTHANASIA</b>	X			
<b>SECTION IV - PHYSICAL PLANT</b>				
<b>13. FUNCTIONAL AREAS</b>	X			
<b>14. CONSTRUCTION GUIDELINES</b>				
a. CORRIDORS	X			
b. ANIMAL ROOM DOORS	X			
c. EXTERIOR WINDOWS	X			
d. FLOORS	X			
e. DRAINAGE	X			
f. WALLS	X			
g. CEILINGS	X			
h. HEATING, VENTILATION, AND AIR CONDITIONING (HVAC)	X			
i. POWER AND LIGHTING		X		
j. STORAGE AREAS	X			
k. NOISE CONTROL	X			
l. FACILITIES FOR SANITIZING MATERIALS	X			
<b>15. FACILITIES FOR ASEPTIC SURGERY</b>	X			
<b>REMARKS</b>				
3a. Risk assessment documentation partially complete. 4a. Room appears overcrowded/cluttered. 4f. Light flickering in Room 3. 14i. Light cover cracked in Room 15.				

DD FORM 2856 (BACK), AUG 2002

Figure D-1. Sample completed DD Form 2856—Continued

### **D-3. Evaluation guidelines**

The DOD Semiannual Program Review/Facility Inspection Checklist was created using the National Research Council's 1996 *The Guide for Care and Use of Laboratory Animals* (Guide) as a template. Refer to the corresponding section of the Guide for more information on evaluation guidelines.



## **APPENDIX E**

### **U.S. GOVERNMENT PRINCIPLES FOR ANIMAL USE**

# U.S. Government Principles for Animal Use

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Interagency Research Animal Committee's

## U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species. Whenever U.S. Government agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or sponsor such procedures, the responsible institutional official shall ensure that these principles are adhered to:

- I. The transportation, care and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.<sup>1</sup>
- II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
- III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.
- IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
- V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
- VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
- VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
- VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their inservice training, including the proper and humane care and use of laboratory animals.
- IX. Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal research committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

<sup>1</sup> For guidance throughout these Principles the reader is referred to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

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Technology Policy

## **APPENDIX F**

# **BENEFITS OF DoD INTRAMURAL AND TRAINING PROGRAMS THAT USE ANIMALS**

# **BENEFITS OF DoD INTRAMURAL AND TRAINING PROGRAMS THAT USE ANIMALS**

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## **Alternatives to Animal Research, Breeding Programs (A1, A2, B)**

- Specific pathogen-free nonhuman primate colonies
- Laboratory technicians properly trained in animal handling and protocol procedures
- Development of a definitive and safe anesthetic regimen for chinchillas used in biomedical research

## **Clinical Medicine (C1)**

- Study of the use of vasopressors in spinal shock/trauma
- Assessment of a new imaging agent (“Acutect”) to detect atrial thrombus formation and cardiac injury due to secondary pulmonary emboli
- Research on the development of a HIV vaccine
- Validation of a treatment regimen for osteoarthritis
- Development of a reliable and nonsurgical method (auditory brainstem response) for determining hearing measurements
- Additional insight into mechanism of cellular damage in muscular dystrophy
- Development of a new rat model of hypertension associated with type II diabetes
- Development of a more effective and rapid method of restoring body temperature to victims of hypothermia
- Better understanding of the development, diagnosis, and treatment of colon carcinomas
- Expansion of basic science knowledge in leukocyte physiology
- Greater understanding of the effects of hyperbaric oxygen on focal brain contusions
- Efficacy testing of a new fibrin sealant bandage to deliver high dose chemotherapy to locally advanced prostate cancer
- Research leading to understanding the etiology of schizophrenia and therapeutic approaches for civilian and military patients
- Research on the effects of a combined-treatment approach to repair chronic spinal cord injuries
- Information on gender differences in nicotine’s behavioral and psychological effects
- Increased knowledge of pharmacological treatments and prevention strategies for neuropsychiatric disorders such as Post Traumatic Stress Disorder in military and civilian victims

## **Clinical Surgery (C2)**

- Four Investigational New Drug Applications awarded by the FDA (1 for phase 1 study of anti-CD154 in human volunteers, 2 for phase 2 trials with anti-CD154 in islet and kidney transplantation, and 1 for phase 1/2 study of anti-B7 antibodies in human renal transplantation) and transitioned into ongoing clinical trials
- Identification of a potential enzymatic, nonsurgical method of ear deformity recontouring
- Design of an invasive carcinoma surgical model to evaluate chemotherapeutic agent using fibrin adhesive
- Research and testing of a corneal implanted optical device to permit limited vision in severe cataract patients
- Studies of a blood substitute to be used in treating hemorrhagic shock following trauma with brain injury
- Provides military physicians with the opportunity to develop and perform surgical research
- Development of a more effective and efficient methodology for treating “empty eye socket” situations in growing children
- Better understanding of appropriate treatment of blood loss shock in the presence of traumatic brain injury using plasma replacements
- Research on skin transplants

### **Infectious Diseases (M2)**

- Identification of two highly effective dengue vaccines
- Development of rapid diagnostic tests to identify caries
- Development of arboviral diagnostic assays for diagnosing dengue, Japanese encephalitis and Chikungunya elicit
- Determine that Shiga Toxin (STX) and other STX family members are potential biological warfare/terrorist threats
- Patent awarded: Ralls, S.A.; Rapid Immunoassay for Cariogenic Bacteria, U.S. Patent No. 6,015,681
- Development of an ELISA standard to measure the mucosal immune response to specific antigens
- Determine cause of up-regulation in apoptotic cells with neuronal morphology
- Testing of GMP Shigella vaccine products for immunogenicity, safety, and efficacy
- Studies and experiments addressing issues in infectious diseases such as malaria, HIV, and diarrheal disease, scrub typhus; ebola, gonorrhoeae

### **Medical Chemical Defense (M3)**

- Maintain control of seizure activity with the use of advanced anticonvulsant treatments
- Discovered that doses of midazolam are efficacious against status epilepticus seizures
- Research on the mechanisms of action and physiological reactions of chemical agents
- Development of a Decision Tree Network for active topical skin protectants consisting of three testing modules that include in vitro, in vivo, and advanced testing

### **Medical Biological Defense (M4)**

- Development of monoclonal antibodies specific to biological/chemical agent stimulants, environmental contaminants and biological toxins
- Development of a model to test the ability of Brucella vaccines to protect against infection following respiratory exposure to *Brucella melitensis*
- Demonstrated and characterized the development of bronchopneumonia, enanthema, exanthema, and consistent monocytosis
- Evaluate early stages of *Bacillus anthracis* spore infection
- Identification of attenuated vaccine candidates for Western Equine Encephalitis and Venezuelan Equine Encephalitis-IE viruses

### **Human Systems Technology (M5)**

- Better understanding of treatment and prevention of “altitude sickness.”
- Production of recombinant and monoclonal antibodies for the development of rapid diagnostic/detection assays
- Development of a rat model to evaluate vascular permeability
- Laser studies permitted the establishment of exposure guidelines for both the military and private sectors
- Assessment of potential hazards and health risks of pulsed microwave radiation, in order to provide for safe electromagnetic environment for military personnel and define safe operation limits for irradiating military equipment
- Research on the effects of single versus multiple subthreshold blast overpressure exposures to lungs, heart, brain, kidney, liver, and gastrointestinal tract

### **Combat Casualty Care (M6)**

- Establishment of a model of combined traumatic brain injury and hemorrhagic hypotension
- Research on the mechanism of mucus genes response to smoke inhalation
- Enhancement of the ability to control lethal hemorrhagic shock with the development of new hemostatic dressings and pharmacologic agents

- Providing surgeons with a real-time imaging tool to visualize thermal injury depth
- Research on resuscitation fluids and documentation of their benefits and side effects
- Investigation of potential treatment modalities for the stabilization of battlefield casualties at high risk of early death to profound hemorrhage and reduction in circulation

#### **Ionizing Radiation (M7)**

- Identification of protection against and treatment of radiation injury

#### **Other Medical RDT&E (M8)**

- Development of cleanup levels for toxins in soil and water
- Research on the mechanisms of human chronic fatigue syndrome
- Quantification of munitions compounds wildlife toxicity

#### **Physical Protection (N1)**

- Updating of the national and international laser safety standards

#### **Other Non-Medical RDT&E (N4)**

- Determination of the requirements, capabilities, and limitations of marine mammals use in operational Fleet Marine Mammal Systems
- Research on the bio-physical properties of the dolphin sonar capabilities and bio-mechanics
- Identification of environmental and human health risks factors
- Toxicological hazard evaluation of chemical threats
- Development of biomonitoring systems to evaluate source water quality

#### **Training, Education, and/or Instruction of Personnel (T1)**

- Increased medical readiness of assigned personnel by refining technical skills and surgical proficiency
- Training physicians in surgical techniques such as cardiovascular surgery, pediatric microsurgery, emergency surgery, obstetrical surgery, vascular and microvascular surgery
- Compliance with 9 CFR (the Animal Welfare Act regulations) where in research personnel are adequately trained and certified to perform animal procedures under controlled conditions prior to working on other approved protocols
- Training in life-saving measures for use in both combat and non-combat situations for health care providers